THE
AMERICAN NATURALIST

VOL. LVIII *January-February, 1924* No. 654

SOME INVESTIGATIONS ON ENTOZOIC PROTOZOA[1]

PROFESSOR R. W. HEGNER
THE JOHNS HOPKINS UNIVERSITY

FIVE years ago a School of Hygiene and Public Health was established as a part of the Johns Hopkins University. One of the divisions of this new school is a department of medical zoology. This is the first department of the kind to be organized in this country, and it has been necessary on this account to determine what actually constitutes medical zoology and what phases of the subject can appropriately be taught and investigated in an institution devoted to preventive medicine. This evening I wish to say a few words regarding medical zoology as a subject and then describe briefly some of the investigations we have carried on in the department on entozoic protozoa.

At the present time the department consists of four divisions, devoted, respectively, to protozoology, helminthology, medical entomology and the filterable viruses. The filterable viruses are agents of disease about which we know very little. They are included but tentatively in the field of medical zoology until we know more definitely what their real status is. The term medical entomology does not exactly indicate the field covered by this subject, inasmuch as not only insects but other arthropods, such as mites and ticks, are included. From the vast assemblage of arthropods those particular species are se-

[1] A lecture delivered at the Marine Biological Laboratory, Woods Hole, Mass., August 3, 1923.

lected that serve as vectors of disease-producing organisms or are actual parasites themselves. Helminthology is likewise a subject that includes forms selected from various groups of animals, especially flatworms and roundworms, that are of medical or veterinary interest. Among the more important of the parasitic worms from the standpoint of public health are the several species of hookworms, flukes, ascarids and filariae.

The teaching of medical zoology differs in no essential feature from that of general zoology. The material to be taught must be selected just as material is selected for the teaching of embryology, invertebrate zoology or physiology, and similar methods are used in presenting the subject-matter to students. Investigations in medical zoology are also similar to those carried on in other fields. They, however, not only involve fundamental biological problems but also frequently have a more or less intimate relation to preventive and curative medicine. It is, therefore, not unusual for a medical zoologist to see the results of his investigations translated almost immediately into terms of human welfare.

Often the terms medical zoology and parasitology are used synonymously. Most of the arthropods of medical importance, however, are not parasites, but are transmitters of disease-producing organisms such as bacteria, protozoa and filariae, and a large proportion of the protozoa referred to in books on parasitology are entozoic but not parasitic. It seems best, therefore, to substitute the term medical zoology for the more common term parasitology.

Many of the most complete accounts of medical zoology are to be found in reference books on tropical medicine, and often as much as one half of such books is devoted to this subject. Much of this space is given over to a zoological treatment of the organisms discussed, thus emphasizing the relative importance of this phase of the subject. We are accustomed to think of many of the diseases that are caused by animal parasites as tropical in their distribution, and it is true that temperature condi-

tions and lack of sanitation make the tropics a favorable environment for the multiplication and spread of these organisms and thus lead to mass infections that bring about the appearance of clinical symptoms. But many of these diseases were once prevalent in temperate regions and even now exist to a certain extent in colder portions of the earth. Their almost complete absence from these regions is largely due to the application of our knowledge of prevention. Some of the most conspicuous successes in the field of preventive medicine have resulted from attacks on diseases due to animal parasites in tropical countries, these organisms lending themselves more readily to preventive measures than most of the other agents of disease.

The protozoa have been favorable material for investigation ever since their discovery, and many of the greatest figures in the history of biology devoted their time largely to this group of organisms. At first free-living species were widely studied and monographed, and only comparatively recently have entozoic forms attracted particular attention. This has resulted from the discovery that certain diseases of man and of lower animals are due to the presence of pathogenic protozoa. Medical men as well as zoologists have carried on investigations on these entozoic species, and medical literature as well as the literature of zoology is in consequence abundantly supplied with the results of these researches. Because of their economic importance certain species are emphasized and others of equal importance, biologically, neglected. However, in almost every case what we know about the species pathogenic to man is based, first, on our knowledge of free-living forms, and second, on related species that occur in lower animals.

In the School of Hygiene and Public Health we consider it appropriate to carry on investigations on all the three types of protozoa just mentioned, namely, free-living species, species entozoic in lower animals and species living in man. Our attention, however, is particularly directed to the study of the entozoic forms and I

shall now attempt to present briefly some of the results of our investigations with these organisms.

For the sake of convenience, we can separate the forms to which I wish to refer into blood-inhabiting species and intestinal inhabitants. Among the most interesting of the former are the malarial parasites and the trypanosomes. The methods of malarial control are comparatively simple and well known, but many important problems still remain to be solved. Of these, one of the most interesting is the problem of relapse. A person who has been infected with malarial organisms by the bite of a mosquito, experiences symptoms as soon as a sufficient number of organisms become present in the blood as a result of asexual multiplication. If the resistance of the host is not sufficient to prevent continued multiplication of the organism, death eventually results. If, however, the patient recovers either with or without treatment, he usually still harbors parasites within his body, which may at some later date again become so numerous as to bring about the recurrence of symptoms, a condition known as a relapse. There are various theories to account for relapses in malaria, one of which we believe we have finally proved. It is difficult to study the subject in the human host; but malarial organisms similar to those in man also occur in birds and can be cultivated in canaries with ease. Drs. Whitmore and Ben-Harel have spent several years investigating the phenomena of relapse in these birds. Apparently in most cases a bird when once infected remains infected throughout its entire life. Clean birds are infected by injecting a small amount of blood from a diseased bird into the breast muscle or peritoneal cavity. Parasites begin to appear in the peripheral blood within about two weeks. They increase rapidly in numbers for a few days and then as rapidly decrease until none can be discovered even after very careful search over a long period of time. The blood of such a bird, however, is still infective to clean birds, and Whitmore was able to inoculate successfully clean birds with the blood of a canary 29 months after the original infec-

tion. It is evident that organisms in some stage in their life history are present in the peripheral blood during this entire period. They may be asexual stages that are too few in number to be found in ordinary preparations, or there may be a stage still unknown in the life history of the organism that has not been brought out by our present method of study. In what part of the host the parasites are located during the intervals between relapses and in what stage of their life cycle are questions that have recently been answered by the researches of Dr. Ben-Harel. She has found that during the period when parasites are apparently absent from the blood, they exist in small numbers in the spleen and bone marrow where they undergo asexual reproduction in a normal fashion. Presumably asexual reproduction in certain of the internal organs proceeds throughout the interval between relapses, the young parasites being liberated from time to time in small numbers into the blood. Conditions favorable to the organism may result in a sudden rapid increase in their numbers, thus bringing about the clinical symptoms which we know as a relapse. With these facts thus well established it is possible to conduct experiments with therapeutic agents designed to attack the parasites that are located in the spleen and bone marrow and are apparently immune to attack by quinine as now employed as a preventive and curative agent. Professor Boyd is now at work on this problem, using the organisms of bird malaria as they exist in canaries and subjecting them to various quinine derivatives kindly prepared for us by Dr. Jacobs of the Rockefeller Institute for Medical Research.

Other problems in malaria that are being investigated by members of the Department of Medical Zoology of the School of Hygiene and Public Health concern the relation between the parasite and its mosquito host. Before any malarial campaign can be carried on with success, we must determine by experiment what particular species of anopheline mosquito acts as a transmitting agent in each particular locality. We must determine what we may

call the biology of these species in order that effective methods of elimination can be carried on. These and other problems of mosquito control are now being investigated at Leesburg, Georgia, by Drs. Darling and Root.

The second group of blood-inhabiting protozoa of great medical importance contains the trypanosomes. The trypanosomes are responsible for the two types of African sleeping sickness, for Chagas's disease, which is prevalent in certain regions of South America, and for diseases in various domesticated animals throughout the world. Studies have been carried on in our laboratory with trypanosomes of frogs, salamanders, rats, man and various domesticated animals, and with several of their intermediate hosts. The first trypanosomes ever described were discovered in fish. Soon after this discovery the frog was found to be parasitized by this type of protozoon. But it was not until these organisms were known to be the causative agents of diseases of domestic animals and later of man that they really came into prominence as objects for research. Our investigations have been concerned principally with the genetics of these organisms and the resistance set up by the host to experimental infections. *Trypanosoma diemyctyli* that occurs in the common newt, *Diemyctylus viridescens,* was the first form studied by us. All the organisms present in the newt, at least in the month of May, appeared to be adults. Of particular interest was the discovery that constant differences occurred in the length of the trypanosomes occurring in different hosts. It seems probable that the trypanosomes from different hosts represent races that are heritably diverse in size, although this difference may be due to differences in the environment as represented by the blood stream of the different hosts.

More elaborate investigations have been carried on by Dr. Taliaferro with trypanosomes in rats. Pure lines of this species, *T. lewisi,* were obtained by inoculating clean rats with single trypanosomes. Infections started in this way indicate that reproduction of the trypanosomes in the blood of the rat ceases within 25 days after the or-

ganisms begin to appear in the blood. From this time on, practically no further reproduction or growth occur and only adult trypanosomes are present. On the basis of these observations an attempt was made to determine whether growing the same pure line in host rats of the same species results in significant differences in size, and whether these differences are similar to those obtained when grown in different species of rats. A statistical study of the data indicates that significant differences of about the same magnitude occur in both cases, but whether these differences are due to heritable diversities or to differences in environment has not been determined.

Another result obtained from these data is that there is no significant change in the coefficient of variation when the pure line is passed from rat to rat by blood inoculation. In contrast to this is the fact that the pure line which does not change in variability when passed through rats increases in variability when passed through its natural insect vectors, the rat fleas. The results indicate that the pure line breaks up during its passage through the invertebrate host, due probably to some nuclear phenomena which may be either the result of a sexual process or of reorganization. No processes, however, such as conjugation or endomixis, have yet been demonstrated in the life cycle of the trypanosome.

As an outcome of this genetical work, Dr. Taliaferro has undertaken an intensive investigation of the resistance which the rat offers against an infection with *T. lewisi*. He finds that there are three manifestations of such a resistance: (1) The rate of reproduction of the parasites is retarded more and more until it is finally inhibited altogether by about the tenth day; (2) a large number of parasites are destroyed between the tenth and fourteenth days; (3) all the rest of the trypanosomes, which exist in the blood as non-reproducing adults, are destroyed from a week to several months afterward (end of infection). The differentiation between that type of resistance which inhibits reproduction and that type which destroys the organisms after they are formed was

made possible by a new method of measuring the rate of reproduction which is irrespective of the number of organisms destroyed. The method consists essentially in comparing the coefficient of variation for some factor involving size and is based on the well-known fact that a sample taken on one hand from a population undergoing rapid reproduction, with the constant production of young forms and intermediate growth stages, will exhibit much greater variability in size than a sample taken on the other hand from a population in which there is little or no reproduction and in which all the organisms are full-grown adults. Results now in press demonstrate that the first manifestation of resistance (the inhibition of reproduction, mentioned above) is brought about by the formation in the blood of infected rats of a reaction product which has the specific property of inhibiting reproduction in the trypanosomes but which does not affect their vitality. This reaction product differs from those known at the present time. To date no mechanism of the second effect of resistance, *viz.*, the first drop in numbers, has been ascertained, but preliminary experiments on the third effect, *viz.*, the total destruction of the organisms, indicate that it is brought about by the formation of a lysin. Work with various pathogenic trypanosomes in different hosts indicates that although, in some, there seems to be a manifestation of resistance similar to the second effect in *T. lewisi,* there is never any inhibition of reproduction.

The second large group of entozoic protozoa to which we have directed our attention contains inhabitants of the intestine of man and of lower animals, and includes genera of flagellates, ciliates and amoebae. These organisms can be studied as they occur in their hosts, but it always facilitates investigation to be able to cultivate the species, with which we are working, outside of the body, especially when the investigation involves experimental studies.

Several common intestinal protozoa have been cultivated for the first time in our laboratory. *Chilomastix*

mesnili, which is a flagellate often associated with intestinal disturbances and which is present in about 4 per cent. of all human beings, was obtained in cultures by Dr. Boeck and could apparently be transferred from one culture tube to another indefinitely. A second species of human intestinal flagellate that has been cultivated for the first time is *Embadomonas intestinalis.* Dr. Hogue succeeded in rearing this species on simple culture media and found that encystment took place under cultural conditions, this being the only species of human intestinal flagellate thus far recorded that encysts in cultures. A third species of intestinal flagellate from man, *Trichomonas hominis,* may likewise be cultivated in simple media. We have on a number of occasions obtained pure lines of this form by inoculating a tube with a single specimen. Trichomonads may possess three, four or five flagella, but when a pure line is obtained all the progeny are provided with the same number of flagella as the original progenitor, indicating that the number of flagella is a heritable character.

The perfection of methods of cultivation has made it possible to undertake investigations of several types. In the first place it provides a more practical and efficient method of diagnosis. The usual procedure in making a diagnosis is to prepare a smear of the suspected fecal material and examine this for flagellates. Dr. Becker and I have found, however, that placing a similar fecal specimen into a culture tube where it is allowed to remain for several days before examination is a more certain method of detecting the presence of these organisms. The perfection of this method will in the future enable us to make more accurate surveys of intestinal protozoa. The effects of external factors on intestinal protozoa can also be determined by studying the results of modification of the culture medium, and work of this sort is now in progress.

With the exception of *Trichomonas hominis,* the various protozoa mentioned are known to form cysts which serve to maintain the race during the period of transfer

from one host to another and to withstand the secretions of the alimentary canal during the passage from the mouth to the intestine of new hosts. From the standpoint of prevention it is important to know something about the longevity of these cysts under various environmental conditions and to determine in what way cysts are disseminated. Dr. Boeck has shown that all the environmental conditions that the cysts are apt to encounter, except drying, can be met successfully by them, but that cysts live for a greater length of time when they are free from the bacterial mass in which they are embedded. Liquids such as water and milk are thus shown to be especially favorable for their distribution. It is generally supposed that cysts find their way from one host to another in drinking water and food that is contaminated and that this contamination is often accomplished by house flies. Dr. Root has been able to prove by a long and careful series of experiments that cysts are easily taken into the alimentary canal of house flies and pass through the body of the fly without injury. It is thus possible for flies to become contaminated and to spread this contamination to food on which they may chance to alight. There can be no doubt that this actually does happen in nature.

Conditions prevailing during the late war stimulated a widespread interest in intestinal protozoa, and besides adding to our knowledge several species that were hitherto unknown, gave us some idea of the extent of the infection with these organisms among human beings. Dr. Payne and I compiled statistics from over 35 papers published by American, English and French investigators, which recorded data from about 20,000 persons. Twenty per cent. of these were infected with intestinal amoebae of the species *Endamoeba coli,* 9 per cent. with *Endamoeba histolytica,* 12 per cent. with the intestinal flagellate, *Giardia lamblia,* 4 per cent. with *Chilomastix mesnili,* and 3 per cent. with *Trichomonas hominis.* So high is the percentage of infection that we usually have no trouble in obtaining material for study or research from some member of the department. It is

probable, even, that among those who are here in this room this evening, there are perhaps 60 infected with *Endamoeba coli,* 30 with *Endamoeba histolytica,* 35 with *Giardia intestinalis,* 12 with *Chilomastix mesnili* and 9 with *Trichomonas hominis.* Fortunately, these organisms are not usually pathogenic or at least the results of their presence are so slight that clinical symptoms do not appear unless enormously large numbers are present.

What effect the changes in the diet of the host might have on the incidence, distribution and numbers of these intestinal protozoa is a question that I have attempted to answer on the basis of experimental work with tadpoles and rats. Particular attention was at first directed toward the problem as presented by the opalinid ciliates in the green frog. These opalinids, at least in certain localities, occur in 100 per cent. of green frog tadpoles but are absent from the adults. This is in contrast to the condition that exists in certain other Anura, for example, in the leopard frog and the common toad. In these forms the infection with opalinids is continuous from tadpole to adult. Dr. Metcalf has suggested that the disappearance of these ciliates in the case of the green frog might be due to the change from a vegetable to an animal diet. Tadpoles, however, that were kept in the laboratory and fed on a strict animal diet did not lose their opalinids until the time of metamorphosis. During metamorphosis not only these experimentally-fed tadpoles but controls collected in the field also lost their opalinids. Apparently, therefore, the change from a vegetable to an animal diet is not the controlling factor. It has been known for many years that metamorphosis in tadpoles is accelerated when thyroid gland material is used as food, and there is some evidence that the presence of some substance in the thyroid may accelerate growth and asexual multiplication in certain free-living ciliates. No increase in the number of opalinids, however, was found to occur in tadpoles of the green frog when fed on thyroid substance, but metamorphosis was hastened and as metamorphosis proceeded, the opalinids

disappeared. It thus seems certain that changes in the digestive tract of the green frog at the time of metamorphosis are responsible for the loss of opalinids and not changes in the diet of the host and that thyroid substance does not accelerate the growth and division of these organisms as it has been supposed to do in free-living species. Why reinfection of the adult green frog does not occur is still unknown.

The intestinal protozoa of the rat to which particular attention was directed are *Giardia muris, Hexamitus muris* and *Trichomonas muris.* I shall refer this evening to the third named species only, *Trichomonas muris.* This species is usually present in great abundance in the cecum of the rat, the material of the cecum frequently consisting almost entirely of a wriggling mass of these organisms. Among a large number of rats obtained from the rat colony of Dr. McCollum that had been fed on various experimental diets, three were found that were free from all intestinal flagellates. The history of these three rats showed that both they and their parents had been fed exclusively on a carnivorous diet. Experiments were initiated on the basis of this discovery that gave striking results in a remarkably short period. Control rats from the colony maintained by the Department of Medical Zoology were found to be 100 per cent. infected with *Trichomonas muris.* These rats had been fed on a diet largely vegetable in nature. Twenty rats from this colony were fed on a well-balanced carnivorous diet for one week and were then sacrificed and examined. Only one of these rats seemed to be entirely free from *Trichomonas muris,* but the number of organisms decreased almost to the point of extermination in the other nineteen. The method of counting used was to take a measured amount of material from the cecum, dilute it with a measured amount of normal saline solution, spread this out under a cover glass 22 mm square, and then count the average number of organisms in ten fields, using a number 10 ocular and a 16 mm objective. It was found by this method that the average number of specimens

of *Trichomonas muris* per field in control rats was 90, whereas an average of less than 2 per field appeared in the rats fed on a carnivorous diet for one week. These results indicate that a carnivorous diet brings about a change in the environment within the cecum of the rat that is very unfavorable to the flagellates. Studies of the hydrogen-ion concentration of cecal material in experimental and control rats indicate that changes of this nature are too slight to account for the results. The rat has been a favorable object for the study of the effects of various diets, and at the present time the explanation that seems best to fit the results obtained by my experiments is based on the character of the bacteria and of the products of their activity. Cannon, for example, has recently shown that when rats are fed on a vegetable diet, intestinal bacteria are present in the ratio of about one of the colon type of 99 of the acidophilus type, and that when a carnivorous diet is substituted for the vegetable diet, this ratio is reversed within a few days to about 99 of the colon type to one of the acidophilus type. The sudden change from a preponderance of the acidophilus type of bacteria to a preponderance of the colon type seems, at present, to be the principal cause of the disappearance of the flagellates. Since these experiments were completed, I have been in communication with three investigators who have carried on similar work with the intestinal protozoa of rats and whose results confirm mine.

Flagellate diarrhoea is a very obstinate disease which often leads to conditions that result fatally. No successful treatment has ever been devised for its alleviation. These experiments on rats, however, suggest that a carnivorous diet for a short period might be an effective method of treatment. This method has actually been put into practice at one of the largest clinics in this country and although up to the present time only a few cases have been available, the effects of the treatment have been remarkably rapid and satisfactory. Inasmuch as one in-

vestigator found that the amoeba of the rat are adversely affected by feeding the host on a carnivorous diet, it seems probable that amoebic dysentery in man may also be successfully treated by proper changes in the diet.

One of the most complex of all biological relationships is that between an entozoic species and its host, and many efforts have been made to determine the evolutionary stages that have ended in parasitism. Various types of association between animals are known. Certain of these involve temporary associations that may even be unnecessary for the successful fulfillment of the complete life cycle of the organism. Other associations exist that are temporary but necessary, and from this point on all stages of association are known ending in a fixed parasitic existence. Many students of parasitic animals have speculated regarding the various stages in the evolution of the organisms in which they were primarily interested. While these speculations are legitimate, the conclusions reached can be of very little value without more thorough observation and experiment as a basis. In contrast to these speculations are the painstaking investigations, such as those on the opalinids recently published by Metcalf, which bring together a vast fund of information, enabling the investigator to reach conclusions based on facts.

Recently an association came to my attention which promises to be favorable for the study of the evolution of parasitism. On a number of occasions during the past decade, I have observed living flagellates of the euglena type in the intestinal and rectal contents of frog tadpoles. They were always considered merely accidental visitors that had been ingested with the food of the tadpoles and were either immune to the digestive juices and were on their way through the intestine, or had not yet succumbed to digestion. Last year, however, these organisms were found in such abundance in certain tadpoles that it occurred to me they might be normal inhabitants of the intestinal tract of these animals. It was first discovered that they are different from any

other members of the Euglenoidina, in that they possess three flagella instead of the usual one or two. It was then proved that they are not digested by the tadpoles nor simply on their way through the alimentary canal, since tadpoles that were starved for 30 days continued to retain in a flourishing condition apparently every specimen originally present. Tadpoles of the green frog, leopard frog and toad were found to be infected, and various experiments proved that uninfected tadpoles of any one of these species could be infected by feeding them active trophozites from the intestine of either of the other two species. Apparent these euglena-like entozoic flagellates are non-specific with respect to these three species of hosts. A striking feature of these organisms is their retention of green chromatophores and red eye-spots while living within the digestive tract of the tadpole. It was found, however, that the color of both chromatophores and eye-spots became somewhat faded in specimens living in the intestines of toad tadpoles. Apparently sufficient light passes through the semi-transparent body wall and intestinal wall of the tadpoles of the green frog and leopard frog to insure the maintenance of the color, whereas the extensive pigment in the body wall of the toad tadpole prevents the entrance of light. By a curious coincidence Dr. Wenrich began a study of these organisms at about the same time that I did. His time was devoted largely to their cultivation and division and his studies indicate that there are two varieties, one green and the other colorless or nearly so and that the green form may transform into the colorless one.

In order to determine whether other types of Euglenoidina could maintain their existence in the digestive tract of the tadpole, I fed uninfected animals on several types of free-living euglenae. The first type used was a small species possessing two short flagella. Millions of these were devoured by the tadpoles, every one of which quickly disintegrated in the tadpoles' intestines. In a second experiment, large reddish-colored euglenoids were

fed to tadpoles of the green frog. Specimens of this species were able to withstand the conditions within the intestine, many of them passing through the digestive tract apparently uninjured; but none of them remained in the intestine or rectum more than 24 hours. A third type of euglenoid fed to tadpoles was discovered by Dr. Reynolds living in the bladders of the bladder-wort, Utricularia. Three tadpoles of the green frog were fed 20,000 of these euglenoids; all the euglenoids were immediately destroyed within the intestine. It is thus evident that although this type of euglenoid is able to resist the secretions within the bladder of the Utricularia plant, it can not withstand the digestive juices of the tadpole. Several parasitic species of Euglenoidina have been recorded in the literature. For example, there is a species of Astasia that lives in the digestive tract of a fresh-water nematode and one that lives in the digestive tract of Cyclops. It seems probable that a large number of Euglenoidina may be more or less parasitic in habit and that considerable light may be thrown on the evolution of parasitism in this group by further studies of the relations between free-living and entozoic species.

As already indicated, the entozoic euglenoids of tadpoles seem to be non-specific with respect to their hosts. This probably indicates a recent adoption of the entozoic habit. This condition of non-specificity of host is also illustrated by certain other intestinal flagellates, notably the herpetomonads that live in the intestine of certain flies. Dr. Becker has shown that the herpetomonads of six species of muscoid flies, which have up to the present time been considered distinct species, are morphologically identical. Specific rank for these herpetomonads must therefore be based on differences of host and not on morphological characteristics. However, Dr. Becker has been able to show by cross-infection experiments that the herpetomonads from any one of the six species of flies will infect clean flies of any of the other species. There is thus shown to be no evidence that specificity of the parasite can be based on the character of the host in this

group of flagellates, and we must conclude that these six species of flagellates in reality all belong to one species.

In contrast to the condition observed in the case of these herpetomonads may be cited the observations and experiments of Dr. Simon and myself on intestinal flagellates of the genus *Giardia*. *Giardia intestinalis* is one of the commonest intestinal protozoa of man, being present as previously stated in about 12 per cent. of all human beings. It is accused of being responsible for intestinal disturbances, although this has not definitely been proved. For many years the giardias found in rats, mice and several types of domestic animals were supposed to be one and the same species, and the contamination of the food of man by cysts from rats and mice was supposed to be the common method of human infection. In 1908 Benson recognized three species of giardias, one from man, one from the rabbit and one from rats and mice. A fourth species was described by Kofoid and Christiansen in 1915 from the field mouse. The careful statistical and cross-infection experiments of Dr. Simon have furnished final proof of the actual distinct specificity of the giardias of man and of rats and mice. I have carried on similar studies with giardias from the tadpole, dog, rabbit and guinea pig, and have found certain constant measurable characteristics that prove that the form living in each of these species is of specific rank. Giardias have also been reported from cats, sheep, pigs and birds. Whether these are likewise specific to their hosts remains for further investigation.

Just what the relation is between many entozoic flagellates and their hosts is not well known. In one case, however, we are now able to state definitely that the relationship is one of symbiosis. I refer to the work recently completed by Dr. Cleveland. His investigations were carried on with the intestinal flagellates of the white ants or termites. A study of the various families of termites with respect to their entozoic flagellates revealed the fact that termites that feed on wood are all provided with flagellates. This indicates that some relation exists between the presence of these flagellates and the wood-feed-

ing habit. Grassi claims that heating an infected termite deprives it of some of its intestinal protozoa. Acting on this suggestion, it was found that by incubating termites at 36° C. for 24 hours, *all* the intestinal protozoa could be destroyed without apparent injury to the termite host. These defaunated termites, however, were unable to digest wood and gradually starved to death on what is ordinarily their normal diet. If, however, they were fed on the products of fungus digested cellulose, they continued to live indefinitely without the presence of intestinal flagellates, although still unable to digest wood. Their ability to digest cellulose, which is the principal food material in wood, was regained when they were reinfected with intestinal flagellates. Experiments with a number of species of termites, both those that feed on wood and those that do not, seem to prove conclusively that these flagellates actually digest cellulose within the intestine of the termite and that the termite depends for its nutritive material, and hence for its very existence, upon the products of this digestion. In connection with the necessity of the presence of these flagellates for the continued existence of the termites, it is interesting to note that termites exhibit one of the most perfect methods known of the transmission of entozoic protozoa from one host to another. The young termites are fed by certain members of the colony on so-called proctodeal food. This is material in a partly digested condition that has passed through the intestine of the termite, where it becomes loaded with intestinal flagellates, and is then transferred immediately to the young termites, all of which are thus assured of infection. It thus appears that the termites can not exist without the presence of their intestinal flagellates, and the flagellates of course can not live except within the intestine of the termites; a true case of symbiosis is therefore established.

I have presented to you, I am afraid very inadequately, the results of some of the investigations that we have carried on with entozoic protozoa. We have studied a number of other species of entozoic forms and have also devoted some of our attention to free-living species. Of

the former I may mention the life-history studies of Dr. Hogue on the amoebae living in the oyster and her comparison of these amoebae with tissue culture cells; morphological studies of the human entozoic amoebae, *Iodamoeba williamsi* and *Dientamoeba fragilis* by Drs. Taliaferro and Becker; the cultivation and morphological study of an endamoeba, *E. barreti,* by Dr. Taliaferro, Dr. Barret and Mr. Holmes; the accurate description of cysts of *Endamoeba cobayae,* by Mr. Holmes; my investigation of *Cytamoeba bacterifera* in the red blood cells of the frog; life-history and morphological studies of *Crithidia gerridis* and experimental studies of the relation between insect flagellates and leishmaniosis by Dr. Becker; observations on nuclear division within the cysts of *Chilomastix mesnili* by myself; on nucleo-cytoplasmic relations in *Opalina larvarum* in conjunction with Dr. Wu, and on nuclear phenomena in a balantidium from the monkey with Mr. Holmes. The free-living protozoa thus far used by us as research material include suctoria studied by Dr. Root, and arcellas by Dr. Reynolds and myself.

To most zoologists the organisms to which I have referred to-night are merely names. Nevertheless, they constitute a large and important section of the animal kingdom and furnish material for investigations of great practical and biological significance. Most significant of all, it seems to me, is the study of the relations of the entozoic species to their hosts, involving not only the great problem of the evolution of parasitism but also problems that require excursions into the field of bacteriology, immunology, chemistry and various other sciences. Scientific knowledge must, in protozoology as elsewhere, precede the successful determination and application of control measures. With this in mind, therefore, we have devoted ourselves particularly, as I have attempted to show this evening, to human entozoic species such as the malarial organism, trypanosomes and intestinal flagellates, etc., and have endeavored by carefully controlled experiments to learn something of their relations to their hosts and how such relations may be employed in the field of preventive medicine.

THE STRUCTURE OF THE VERTEBRATE EYE AS AN INDEX OF DEVELOPMENTAL DEFICIENCIES: WITH THE BEARING ON RECENT INHERITANCE STUDIES

PROFESSOR CHARLES R. STOCKARD

CORNELL UNIVERSITY MEDICAL COLLEGE

THE embryonic development of vertebrate animals normally takes place at definite rates. The rate changes during different developmental stages, but is in general typical for the given species. Unusual conditions arising in the environment tend primarily to alter or interfere with the usual rate of development. It is probably also true that changes may take place within the germ-cells or within the embryos themselves which primarily tend to give rise to a new developmental rate. The modification in rate may be limited to certain embryonic periods or may exist throughout development. In any case, should the change in rate be sufficiently marked, it is followed by modifications in various structural formations. The structure to be most modified will vary, depending upon the stage of development during which the change in rate occurs. The organ or part which should at the time be developing at comparatively the most rapid rate is most affected by the particular interruption. The development of the body axis, eyes, ears, mouth, gills and certain of the alimentary glands may be modified with varying degrees of success by treating the embryo at the several critical moments determined for the origin of these structures. It can be definitely shown in almost every case that the modifications in structure result from an alteration in the developmental rate.

When an organ is structurally arrested or altered by a change in the rate of development, it rarely recovers from this modification, even though a normal rate of development be subsequently established. The failure to recover is probably due to a competition of some kind,

which seems to exist among the developing organs of an embryo. When a part has lost its opportunity to arise or develop at the proper time it is unable to attain this aim at a later moment, since other parts have come more or less into developmental supremacy. There may simply be a change in metabolism or rate of oxidation occurring in the several organs at various times of development. For a complete consideration of the effects of developmental rate on the origin and structure of organs in the embryo, the reader is referred to a previous paper on the subject, Stockard ('21).

If the above propositions are correct it follows that any organ or part which develops in a comparatively active manner throughout a long period of embryonic life is particularly liable to injury through changes in rate. The vertebrate eye is distinctly such an organ.

Period and Extent of Eye Development

Briefly surveying the development of the vertebrate eye, it is found to be the first actual organ to arise in the embryo and appears as an enormous outgrowth from the forward end of the neural folds. The appearance of the neural plate is almost the first visible sign of the establishment of the embryonic line or axis and follows closely after gastrulation. The neural plate scarcely begins to form its lateral folds before the anterior end becomes greatly widened, and the first observable step in the origin of the eye occurs. The two great optic vesicles grow out laterally at a rapid rate and invaginate to form the huge optic cups. No investigator can view these processes as they proceed in the living embryo without being impressed with the comparatively great amount of developmental energy necessary to bring about such growths, which in themselves constitute a large fraction of the entire mass of the embryo.

Another neighboring growth of considerable dimensions begins in the ectoderm overlying the optic vesicle, first a thickening, then an invagination of the thickened

area and finally a large, somewhat spherical, mass is constricted away to differentiate into the crystalline lens of the eye. The primary optic cup, or future retina, and the accessory parts of the eye continue an active growth and development, so that even in late embryonic stages the eye still constitutes a great portion of the body, instead of being the comparatively small organ of the final adult head.

Thus the eye arises soon after the primary embryonic axis is established, and its development continues with the differentiation of the retina, the ingrowth of the optic nerve fibers from the retinal ganglion along the optic stalk and the addition and differentiation of numerous accessory parts, lens, humors, glands, muscles and lids. Nothing in developmental dynamics is more striking than these processes, which finally produce the vertebrate eye. And interruptions during development or any deficiency in general developmental energy are, therefore, quite likely to mar the full perfection of the eye in some or all of its parts. It is well known that the eye frequently shows maldevelopment in both its primary and secondary structures, and this fact is to be correlated with its long period of development.

SUSCEPTIBILITY OF THE EYE DURING DEVELOPMENT

The peculiar susceptibility of the eye to developmental modifications may be illustrated by a consideration of specific cases. It has been shown by the writer and a number of others that when the eggs of marine fish are developed in sea-water to which has been added either salts, sugars, substances with anesthetic properties or various other substances that the embryos resulting present a wide range of defective eye conditions. The development of one or both eyes may be entirely suppressed, or either one or both eyes may show all degrees of microphthalmia and maldevelopment. One eye may be perfect and the other absent, or both may be equally defective. It would seem as though one eye frequently pos-

sessed a developmental advantage over the other and that a condition which completely suppressed the right eye, for example, might have no effect on the left. Further, it appears to be quite possible that when only one eye is handicapped the other eye actually profits by the suppression of its mate, since it so often develops unusually well. There is likely some form of competition between the two eyes.

Exactly similar eye conditions may be produced by subjecting the eggs, during early stages, to low temperatures or by reducing their oxygen supply, in both cases slowing the rate of development. The eye response is, therefore, general and tends to follow any treatment that depresses the development of the embryo.

Leplat, '19, has shown the amphibian embryo to respond in a manner similar to the fish when subjected to unusual chemical environments. The eye is frequently abnormal and of the same general types as those cited above.

When the bird's egg is exposed to the action of volatile substances, such as ether or alcohol, I have found, as Féré had previously shown, that enough of these fumes penetrate the shell to affect the developing embryo. These embryos also frequently develop abnormal eyes of exactly the same type as those in the fish. It is thus evident that the eye is a susceptible organ in all classes of vertebrates and may be easily modified by changing the environment of the developing egg.

The mammalian embryo presents a somewhat different case on account of its internal development and, therefore, better protection. But it is found that the female guinea pig may be so treated with ether or alcohol as to affect the developing embryos and cause them to form abnormal eyes. In the case of the female mammal the treatment used may act directly upon the developing embryo if taken into the placental circulation, and the conditions, therefore, become comparable to those acting upon the externally developing eggs of fish, amphibians and birds. Guyer and Smith ('18) have obtained similar re-

sults by treating the pregnant rabbit with fowl serum containing lens antigen.

The problem may, however, be taken a step further by treating the male mammal. We have found that after treatments of sufficient duration and intensity the male guinea pig may sire by normal females young with various defective conditions of their eyes. These animals may be entirely eyeless, one-eyed or with different degrees of microphthalmia, various opacities and abnormalities of the lens, and other types of maldevelopment. The maldevelopment in this case is not due to unusual conditions in the environment surrounding the embryo, since the mother is untreated and is a tested normal animal.

The eye abnormalities of the young sired by a treated male must be attributed to an injury or modification in the germ-cells. The injury has affected the spermatozoon in such a manner that when it fertilizes a normal egg the developmental capacity of the zygote is lowered and a defective individual is produced. It seems highly probable that the chromatin in the germ-cells has been affected and the subsequent generations arising from this injured germ-plasm continues to be inferior as compared with the control generations. The treatments have brought about a general lowering of the developmental capacity of these germ-cells and they give rise to generations of defective and under-developed individuals. No specific response has been detected. The effect is general, and the young animals frequently show defects of the eyes closely similar to those induced by slowing the developmental rate of other vertebrate species. One might imagine that the treatment had acted to disturb the genes which determine the normal coordination of the developmental processes. There may be certain genes that regulate the rate or activity of development, just as there probably are genes determining the limits of growth. These physiological factors seem disturbed, and the results are general rather than specific for definite structural characters. It might be that groups of genes are affected in a way to disturb the usual develop-

mental processes. The effects are more far-reaching than if a single gene was involved, unless there be only one gene which definitely determines the rate of development.

The treatments affect the germ-cells of the mammal and cause them to give rise to subnormal offspring, and the defective conditions, when once induced, are transmitted in the race until the defective group finally succumbs.

With the treatments we have used on the guinea pig, only part of the germ population would seem to be injured, while the more resistant germ-cells apparently escape, but the details of these experiments are not of importance in the present considerations.

Is there Specific Inheritance of Vertebrate Eye Anomalies?

From the above cited studies of the behavior of the eye during development and the transmission of eye defects in degenerate stocks, we are inclined to believe that any treatment of a mammal which would tend to injure its germ-cells might very probably cause maldevelopment of the eye. Several workers have recently recorded important results of this kind which we may briefly examine.

Guyer and Smith ('18, '20) have carried out an ingenious series of experiments with injections of lens antigen into rabbits. Their method was to crush the crystalline lenses of rabbits and inject this material into chickens and later to collect the blood from the injected chicken. The serum which contains an antigen against the rabbit lens is then administered in definite doses to the rabbit. The doses used are slightly less than a fatal amount; when a little more than the experimental dose is given the rabbit dies. The treatment is very toxic. The antigen does not injure the lenses of the rabbit into which it is injected, but it affects the germ cells of this rabbit and some of its offspring may show defective eyes and cloudy or badly formed lenses. These eye defects are transmitted to later generations and seem to be inherited in

this line of rabbits. Guyer and Smith have, therefore, interpreted the result as a specific response of the germplasm to the lens antigen and hence the defective lenses of the progeny. The facts are extremely interesting, but do they not lend themselves to a different and more probable explanation?

In the first place, the control animals are treated with normal fowl serum and with so-called antigen of rabbit testis which may be somewhat less toxic for the rabbits than is the lens antigen. These control treatments may be only passive substances or their action may not be of the type to affect the germ-cells at all; certainly two different treatments rarely act in a similar way on the same organ.

In the second place, if the lens treatment has a specific action on the germ-cells, why is not the lens of the offspring alone affected? Guyer's specimens show numerous defective conditions of not alone the lens but of the entire eye ball. The eye may be almost absent, as in the alcoholic guinea pig. Or suppose the treatment was specific for not only the lens but for the entire eye—then why do so many of the progeny show defects in only one eye, while the other eye is perfect? It seems somewhat peculiar to inherit a specific eye condition in one eye and not in the other. Yet it must be recalled, as Guyer points out, that certain conditions such as polydactyly, which is definitely inherited, is frequently asymmetrical in its expression.

These eye conditions are, however, exactly what would often obtain from a general injury of the germ-cells, causing a lowered developmental capacity, and just such a condition is transmitted in our defective guinea pig stocks. The eye reactions recorded by Guyer and Smith seem to be much like typical developmental arrests and may not be specific reactions to a definite treatment. They may be genetic in the sense that certain genes are affected by the treatment and are so disturbed as to give improper developmental coordination. The germ is modified so as to be incapable of well-organized develop-

ment and this altered condition of the genes may be transmitted, but the expression which indicates the new condition is general and secondary and is merely the result of a lowered developmental capacity or an arrest.

It is well known that similar eye defects—monophthalmia and asymmetrical microphthalmia—often occur spontaneously in weak or degenerate races of birds and mammals. Such conditions may appear for several generations, and are indicative of general maldevelopment in the stock. But that there is a specific genetic basis for eye defects may be seriously questioned when one considers a type of developmental derangement which I have recently studied, the commonly known double or twin conditions. In double-headed embryos and in united twin specimens the components frequently differ in size and, when they do, the smaller one is invariably deformed. The smaller component has a slower and more irregular developmental rate than the larger, and it shows every kind and degree of eye abnormality that is mentioned above. The lens is frequently abnormal and all grades of microphthalmia occur in either one or both eyes. This type of specimen conclusively demonstrates that the eye defects are the result of a depressed development, since they only occur in the smaller, poorly formed, weak component and not in the larger component which is as typically normal in its structure as a single individual might be. Certainly no defect in the smaller component could be genetic in origin, since the two components are derived from a single fertilized egg and the genetic composition of the smaller component is the same as that of the larger. The double specimen with unequal components serves to illustrate in a most convincing manner the extreme susceptibility of the vertebrate eye to any developmental disadvantage and shows it to be the most reliable and constant indicator of developmental deficiencies.

No other organ or part in the lesser component of the double specimen is so constantly deformed as is the eye. In view of these facts it would seem highly probable that a transmitted defect of the eye is not a specific eye condi-

tion due to a changed eye factor but is far more likely due to a modification or change in those factors in the germ which have to do with a proper developmental coordination and capacity.

Very recently Bagg and Little have reported that after mice are treated with a definite dosage of X-ray a germinal modification is produced which gives rise to maldevelopment of the eye along with several other structural deformities. The specimens showing the abnormal eyes may be selected out and finally bred so as to give approximately 100 per cent. of individuals with abnormal eyes. The condition is inherited in the race as a Mendelian recessive. The effects of the X-ray treatment seem to be much more universal on the germ-cells than either our alcohol treatments or Guyer and Smith's lens antigen, and for this reason Mendelian expectations are more nearly attained. That is, it becomes possible to obtain a purely breeding recessive. In our alcohol experiments it seems very probable that with the treatments used only a fraction of the entire germ-cell population is modified and a certain lot escape. We are thus breeding animals containing a mixture of normal and modified germ-cells and the number of defective animals in each generation is below the Mendelian expectation. Guyer's results seem somewhat the same. Bagg and Little have, however, succeeded in getting with X-ray a modification of the total germ population and their genetic records are far better than ours.

The question still arises regarding the specificity of the eye defects Little and Bagg find. These eye conditions are all of exactly the same nature as those described above as resulting from developmental interruptions.

It is again difficult to think of the eye defects in X-ray mice as being due to a mutation or change in a particular gene determining eye structure. We can certainly not think of the similar eye conditions as having been due to any such specific gene alterations in the directly modified embryos of fish, birds and rabbit. Here again it may be imagined that the X-ray treatment has so modified the

chromatin as to lower its capacity for giving rise to a zygote with a well-regulated normal development. Masses of the chromatin or blocks of genes may be disturbed in some way, and such modified masses may be segregated in a Mendelian fashion. The fact that the F_1 individuals do not show the defects would not argue against such a gross modification of the chromatin, since a non-disjunction or some form of melting together of the genes might occur in a gradual manner and might not be brought about at once in the directly treated germ-cells of the exposed P_1 generation.

It is difficult at present to speculate on an exact mechanism by which these defective conditions are transmitted. And it may be possible that some simple mass action of altered chromatin may be involved. It is true in both Guyer and Smith's rabbits and Little and Bagg's mice that the number of defective individuals increases through several generations before high proportion of such individuals are reached. This might indicate that the eye condition was dependent upon several factors instead of one or only two. But it might also indicate that when the defective lines are closer inbred a larger proportion of modified chromatin is present in each zygote and, therefore, the development is more uniformly subnormal as is the case among a degenerate family of animals.

Blind Forms in Nature

There is a condition widely present in nature which is closely related to the problem under discussion. I refer to the blind and eyeless forms of fish, amphibians and a few reptiles which live in a dark or cave environment. Numerous speculations have been advanced as to the origin or cause of these blind forms, and it may not be altogether harmful to add another, particularly since the foregoing consideration of the susceptibility of the developing eye tends to substantiate the view to be presented.

All the blind vertebrates living in dark and protected places have close relatives with perfectly developed eyes

living in the light. The blind form in every case, I believe, is a weaker, less well-developed animal than its nearest relative. The more hardy and active form is never the cave-living one. It might be suggested that the blind animal is less active because of its blindness, but if the blindness be due to poor development then a low activity would be correlated with it.

The embryos of all blind vertebrates that have been studied show that the optic vesicles, and often lenses, are formed and then become arrested, and either completely degenerate or persist as maldeveloped eyes buried in the head. Those forms that merely burrow and hide temporarily under cover, such as Rhineura, the Florida burrowing lizard, exhibit the greatest variation in the degree of degeneration of the eyes. Eigenmann noted that the lens was absent from 50 per cent. of the eyes of Rhineura and was variable when present. Such animals are not forced to seek a permanent abode in the dark. Other forms that live constantly in the dark of deep caves show a more uniformly degenerate eye with little variation in degree of development.

These blind animals may not have inherited a specific eye character, but may rather have inherited a more or less definite change in their developmental rate or capacity during a period peculiarly critical for the developmental expression of the eyes. A single mutation may have been responsible. The darkness has not on any substantial ground been shown to be a causal factor in such conditions. If these animals represent a genetic type of subnormal development then it is rather logical to presume that they will timidly seek cover and come to reside in dark places, while the bolder and more fully developed near relative would have no such tendency to seek the shelter of caves.

CONCLUSION

Finally, then, there is remarkable uniformity of type and condition in the maldevelopment of eyes, from direct injury of the egg or embryo, in the offspring of mam-

mals with modified germ-cells as a result of various treatments, and under natural environments. The eye is definitely a susceptible and responsive organ during development. In view of this it seems a safe attitude to consider the vertebrate eye as an indicator of developmental deficiency and to question the specificity of origin of any eye anomaly. It may further be appreciated that when the eyes are structurally deformed or deficient in an individual there is a likelihood that other organs and parts have also suffered from the same arresting cause. This is well shown in many cases by the experimented embryos, our alcohol treated guinea pigs and the X-ray mice of Bagg and Little. Conversely, it is probably more often true that when developmental arrests are present in various body organs, the eyes of such an individual are also structurally deficient.

REFERENCES CITED

Bagg, H. J.
The absence of one kidney associated with hereditary abnormalities in the descendants of X-rayed mice. Proc. Soc. Exp. Biol. and Med., 21, 1923.

Guyer, M. F., and Smith, E. A.
Some prenatal effects of lens antibodies. *J. Exp. Zool.*, 26, 1918.
Transmission of induced eye defects. *J. Exp. Zool.*, 31, 1920.

Leplat, G.
Action du milieu sur le développement des larves d'Amphibiens. *Arch. de Biol.*, 30, 1919.

Little, C. C., and Bagg, H. J.
Proceedings American Society of Zoologists. *Anat. Record*, Jan., 1923.

Stockard, C. R.
Developmental rate and structural expression: an experimental study of twins, deformities, etc. *Am. J. Anat.*, 28, 1921.

A SEXUAL ACTIVITY RHYTHM IN THE FEMALE RAT[1]

G. H. WANG

THE PSYCHOLOGICAL LABORATORY

THE JOHNS HOPKINS HOSPITAL

INTEREST in periodic activity led us to the discovery of a regular four-day rhythm in the variation of the daily amount of activity in the female rat. Four experiments were performed with a total number of nineteen rats—fourteen females and five males. In these experiments, each rat was placed in an activity cage, which consisted of a small living cage and a revolving drum. The revolving drum was attached to the living cage in such a way that the animal in the activity cage had free and easy access to the revolving drum (13 inches in diameter.) A ratchet cyclometer was connected to the revolving drum by a system of levers and registered automatically the revolutions of the drum in both clockwise and counter-clockwise ways. Thus, the daily records of the readings of the cyclometers served as a fairly accurate measurement of the daily variations of the amount of activity. Environmental conditions were very carefully controlled through all the experiments; the animals were kept under constant illumination, in fairly uniform temperature (around 20° C.), and with food and water all the time. The fourteen females and five males experimented on all gave the same results. Only females which had reached sex-maturity showed the regular four-day rhythm, and no male showed any such regular rhythmic variations.

In Fig. 1, a typical activity curve of a female rat is given. The number of the revolutions of the revolving

[1] The full account of this work is going to be published as No. 6 of the Comparative Psychology Monographs.

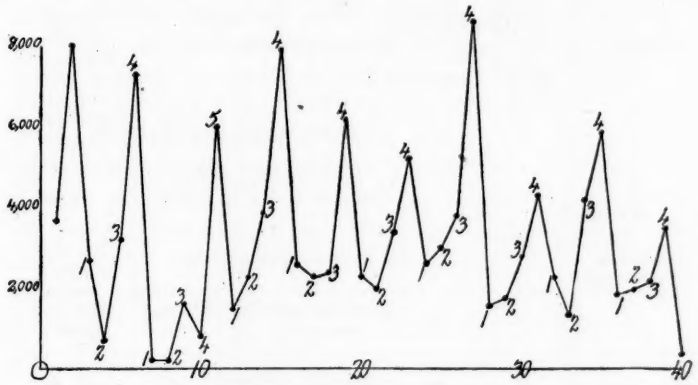


FIG. 1. A typical activity curve of a female rat. Rat D2. Age: 108–148 days.

drum is plotted on the ordinates and the number of days of the experiment on the abscissae. This curve shows many "peaks." If the intervals between these "peaks" are examined, it will be found that nearly all of them are four days in length (with only one exception). The drop in activity after each "peak" is generally very large. It is usually about 60 per cent. and sometimes amounts to more than 90 per cent.

On all the fourteen female rats experimented on, a total of 192 intervals was observed. The mode (98 instances) of the distribution curve of these 192 instances is four days, and the range of variation from two to eleven days.

Attempt was then made to find out whether this regular activity rhythm is related to the oestrous cycle. According to Long and Evans (1), the oestrous cycle in the rat is on the average four days in length, varying from three to thirteen days. The oestrous cycle is divided into five stages, which can be differentiated in a living animal by microscopic examination of smears of vaginal content and by the sexual behavior of the animal. The results of Long and Evans on these points are given in the following table:

Stage	Length in Hours		Cellular Elements of Vaginal Smear	Sexual Behavior
	Mode	Average		
1	12	14.2	Nucleated epithelial cells.	Not in "heat"[2]
2 (period of oestrus)	27	38	Cornified epithelial cells.	*In "heat"*
3 (period of ovulation)			Cornified epithelial cells.	Not in "heat"
4	6	7.8	Cornified epithelial cells and leucocytes.	Not in "heat"
5	48	53	Nucleated epithelial cells and leucocytes.	Not in "heat"

[2] Some animals may go into "heat" in the latter part of this stage.

On the basis of these results, experiments were carried out to find out the time relation between the activity rhythm and the oestrous cycle. It was found that, in fifty cases, the vaginal smear made on the day of the "peak" of activity nearly always showed cornified epithelial cells alone (with only four exceptions, in which cases the smear made on the day before the "peak" showed cornified epithelial cells.) It was also found that the female rats were in "heat" on the day of the "peak" of activity. These findings showed that the activity rhythm bore a definite time relation to the oestrous cycle and that most likely a female rat was active during the period of oestrus. Another experiment with four animals was then performed to determine the exact time relation between the two periodic phenomena. In this experiment, readings of the cyclometers were taken and vaginal smears made every six hours (Noon, 6 P. M., 6 A. M., and Midnight). Each animal was taken out of its cage at these times and offered to a male in an ordinary rat cage. The sexual behavior of the female was closely watched for five minutes, actual copulation being prevented. At the end of this period of observation, the female was returned to its activity cage. The results of

this experiment demonstrated that a female rat is most active during stage two of the oestrous cycle, when vaginal smear shows the presence of cornified epithelial cells alone, and the animal is receptive (see Fig. 2).

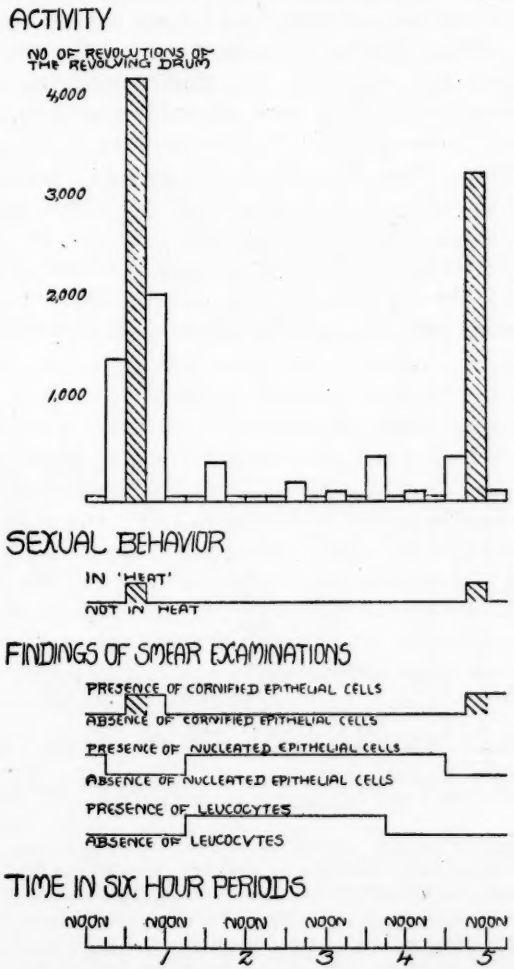


FIG. 2. Graph showing the exact time relation between the activity rhythm and the oestrous cycle. Rat P1. Age: 130–135 days.

Since the activity rhythm and the oestrous cycle are definitely related in the sequence of occurrence, the question arises: "Which one of these two periodic phenomena is dependent on the other?" Several experiments performed bear on this question. For instance, one experiment was carried out with four female rats to determine if the activity rhythm is present during the periods of pregnancy and lactation. In this experiment activity records on each animal were taken for a sufficient length of time to make sure the regular recurrence of the activity rhythm. Then, the animal was mated. Daily records of activity were taken through both periods of gestation and nursing. The litter was weaned on the twenty-first day after birth. All the four animals tried gave the same results. The regular four-day activity rhythm was absent during gravidity and lactation. It is also of interest

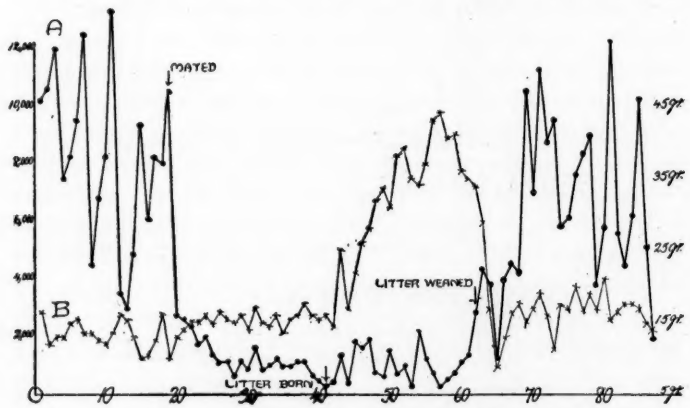


FIG. 3. Curves showing the effects of pregnancy and lactation on activity and food intake.

A = Curve of activity. B = Curve of Food-intake. Abscissae = Course of experiment in days. Ordinates = Number of Revolutions of the revolving drum per day for A; and = Amount of Food-intake per day for B. Rat S3. Age: 90–177 days.

It is interesting to note on the food-intake curve the slight increase of daily food-intake during pregnancy and the sudden increase after the litter was cast. During the period of lactation, the amount of daily food-intake may reach as high as three times the normal amount.

to note that there was a big and sudden drop in activity after conception. The activity level was continuously low throughout both periods of pregnancy and nursing. Daily activity records were also taken for a sufficiently long period of time after weaning to see that the activity rhythm returned, and the activity came back to its normal level. It was found that it took more than five days for the return of the normal level of activity and more than ten days for the return of the activity rhythm (Fig. 3).

A second experiment was carried out with four just weaning females (20 days old) to ascertain whether the activity rhythm was present before sex-maturity. The results of this experiment were: (1) that the activity was on a very low level and without any indication of rhythmic variations when the females were 20–40 days old; (2) that after this there was a period (about 10–20 days) of slight increase in activity and with somewhat irregular rhythm; and (3) that the high "peaks" and regular cycle suddenly appeared. Some experiments made later on showed that oestrous cycle as determined by smear examination appeared in the second period but it was of irregular length, and that high "peaks" and regular activity rhythm came into existence at the same time with the appearance of regular oestrous cycles.

Another experiment deals with the effect of ovariectomy and hysterectomy. The results came out just as expected, that is, activity rhythm ceases with double ovariectomy, but persists with the removal of the two horns and body of uterus. It is also very interesting to note that the ovariectomized females have as low levels of activity as the immature ones.

The main results of the experiments briefly described above are: (1) Adult female rats show regular four-day rhythm in activity; (2) The activity rhythm bears a definite time relation to the oestrous cycle, the animal being most active during oestrus and before the onset of ovulation; (3) The activity rhythm is absent in cases when

the oestrous cycle is not present, for instance, during pregnancy and lactation, before puberty and after ovariectomy.

Then, the conclusion is that this activity rhythm in the female rat is a sex rhythm and depends on the regular periodic functioning of the ovaries.

REFERENCE

(1) Long & Evans. The Oestrous Cycle and Its Associated Phenomena in the Rat. 1922.

INBREEDING THE RHODE ISLAND RED FOWL WITH SPECIAL REFERENCE TO WINTER EGG PRODUCTION (PRELIMINARY REPORT)[1]

DR. F. A. HAYS
MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION

DIVERSE opinions exist concerning the advisability of inbreeding flocks of chickens. The opinion is rather general among poultrymen that low vigor, low hatchability and high mortality, as well as many other faults, may result from close matings. The fact is very well demonstrated that the uniformity that comes from inbreeding is the result of a nearer approach to homozygosity in Mendelian factors. With this greater homozygosity in desirable characters comes the homozygosity for undesirable characters. If the assumption is true that desirable characters—those that have made the perpetuation of the race possible—are dominant, we have a means of explaining why the more heterozygous (those not inbred) are the more vigorous. This may be a means of explaining why flocks deteriorate in some desirable qualities if bred without the introduction of new blood. If the breeder were able to start with a foundation homozygous for these so-called desirable characters, it is not inconceivable that inbreeding could go on indefinitely without any defects occurring.

The question arises: Are the standards for selecting breeding fowls correct to the extent that their application enables the breeder to distinguish the kind of individuals he really wants? May it not be possible that homozygous individuals would not meet the requirements in individuality? Here enter the effects of heterosis. Because of the great complexity of modern breeds of poultry, ideals

[1] Contribution No. 1 from the Massachusetts Agricultural Experiment Station.

may call for a type exhibited only by heterozygous individuals, and when attempt is made to approach homozygosity by inbreeding, the progeny may be lacking in what is called good vigor, high hatchability, etc.

All poultry breeders are striving to establish uniformity in their flocks. Experience teaches that the only known method of securing uniformity is through inbreeding. But while inbreeding for uniformity in color, type, weight, etc., the breeder may be confronted by low vigor, low fertility, high mortality, etc. Such results would at once tend to condemn close breeding. The fact remains that close breeding has been used for one purpose, *viz.*, to secure uniformity. But the uniform individuals do not possess the vigor sought for. This vigor may be had as Wright (1922) has shown in guinea pigs by mating to unrelated stock, but how can uniformity be retained if unrelated stocks are brought together? The method is simple and is the one outlined by East and Jones (1919) for breeding corn: Develop several unrelated inbred strains to a high degree of uniformity for desirable characters of the breed, then cross these and the uniformity remains and renewed vigor results from heterosis.

A preliminary experiment in inbreeding Rhode Island Red chickens was undertaken by Dr. H. D. Goodale at the Massachusetts Agricultural Experiment Station in the spring of 1919. Four crops of chicks have been secured to date. This preliminary report covers the results on birds hatched in 1919, 1920 and 1921 all of which were given an opportunity to demonstrate their winter egg-laying ability.

FOUNDATION STOCK

The birds used in this experiment were all pedigree Rhode Island Reds that had been bred for egg production for six years at this station. One hen No. B357 was mated to her son B3808 in 1919. This hen made a second year record of 172 eggs. She was used for breeding for four seasons and lived to be over five years old. There was no common ancestry in the pedigree of this hen for six

generations, and she was not related to her mate, the sire of B3808. Hen B357 was fully up to breed requirements in size; her pullet year record was 286 eggs, and 73 per cent. of her eggs were fertile the first year with a hatchability of 63 per cent. Males in all cases were selected carefully to secure the best individual where several were available. All daughters were trapnested throughout the winter unless physically unfit. Other hens used during the first year were two full sisters to B357, one distantly related hen and four unrelated hens. All these hens were yearlings and each had a trapnest record.

General Plan of Matings

The general plan was to concentrate the blood of B357. A son of hers by a son was used in 1920. In 1921 two sons by a son of this hen were used. Unrelated hens were used as checks the first year. Female descendants that were full sisters were mated to their full brothers in 1921. The combined results are presented in Table I.

Table I gives results of mating male B3808 to his dam, two of her full sisters and one more distantly related hen in the inbred group. In the check group are four females all unrelated to B3808. A reasonable expectation would be for a considerable degree of uniformity in winter egg production in the daughters of the first three hens because they are full sisters and are mated to a son of one of them. This point will be considered later.

A general tendency for the mean winter egg production of the daughters to fall below their dams is observed. This is to be anticipated for no other reason than that the dams are a selected group and far excel the rest of their full sisters in winter egg yield. In those few cases where the average of the daughters excels the dams, the explanation lies in the effects of factors for high production contributed by the male.

The possible relation between the degree of inbreeding and mean winter egg production is interesting. The 25 inbred daughters of B3808 averaged 56.4 ± 17.50 winter

TABLE I

PRELIMINARY INBREEDING EXPERIMENTAL RESULTS

1919 Hatch

Male Number	Female Number	Winter Egg Record	Winter Rate	Winter Pause Days	Inbreeding of Daughters	Daughters' coefficient of variation for winter egg Production	
B3808	B 357	Dam 112 2 Daughters 50.5	Dam 73% Daughters 67%	Dam 0 Daughters 27	25 % Inbred 62.5 % Homozy.	10.89%	
B3808	B 359	Dam 106 9 Daughters 60	Dam 80% Daughters 47%	Dam 39 Daughters 41	25 % Inbred 62.5 % Homozy.	48.67%	
B3808	B 754	Dam 73 8 Daughters 59	Dam 66% Daughters 52%	Dam 8 Daughters 31	25 % Inbred 62.5 % Homozy.	42.86%	45.34%
B3808	B 699	Dam 80 6 Daughters 42	Dam 64% Daughters 49%	Dam 0 Daughters 28	6.25 % Inbred 53.12 % Homozy.	36.80%	
B3808	B 710	Dam 93 7 Daughters 60	Dam 60% Daughters 65%	Dam 21 Daughters 21	Not Inbred	43.18%	
B3808	B 785	Dam 84 7 Daughters 87	Dam 80% Daughters 71%	Dam 4 Daughters 9	Not Inbred	27.00%	38.00%
B3808	B1357	Dam 72 13 Daughters 58	Dam 67% Daughters 55%	Dam 0 Daughters 20	Not Inbred	32.68%	
B3808	B2219	Dam 39 7 Daughters 50	Dam 65% Daughters 62%	Dam 0 Daughters 16	Not Inbred	36.98%	
			1920 Hatch				
B5806	B 357	Dam 112 5 Daughters 44	Dam 73% Daughters 47%	Dam 0 Daughters 9	37.5 % Inbred 68.75 % Homozy.	46.93%	

TABLE I (*Continued*)

Male Number	Female Number	Winter Egg Record	Winter Rate	Winter Pause Days	Inbreeding of Daughters	Daughters' coefficient of variation for winter egg Production
B5606	B3512	Dam 38 1 Daughter 18	Dam 47% Daughters 18%	Dam 28 Daughters 67	25.00 % Inbred 62.5 % Homozy.	.....
		1921 Hatch				
B8273	B8318	Dam 76 4 Daughters 50	Dam 70% Daughters 53%	Dam 0 Daughters 14	28.125% Inbred 64.062% Homozy.	20.00%
B8273	B9796	Dam 58 1 Daughter 44	Dam 81% Daughters 44%	Dam 0 Daughters 31	28.125% Inbred 64.062% Homozy.	.....
B8273	C 23	Dam 18 4 Daughters 28	Dam 41% Daughters 31%	Dam 19 Daughters 53	28.125% Inbred 64.062% Homozy.	29.11%
B8574	B8318	Dam 76 3 Daughters 13	Dam 70% Daughters 43%	Dam 0 Daughters 8	28.125% Inbred 64.062% Homozy.	23.91%
B8574	B9651	Dam 26 2 Daughters 51	Dam 54% Daughters 62%	Dam 0 Daughters 0	28.125% Inbred 64.062% Homozy.	20.00%
B8574	B9796	Dam 58 1 Daughter 55	Dam 81% Daughters 72%	Dam 0 Daughters 0	28.125% Inbred 64.062% Homozy.	.....
B9275	B 357	Dam 112 2 Daughters 57	Dam 73% Daughters 72%	Dam 0 Daughters 4	43.75 % Inbred 71.87 % Homozy.	9.45%

eggs; the 34 outbred daughters averaged 63.24 ± 15.675 eggs. There is, therefore, no significant difference in the mean winter egg production of the daughters of this male that are inbred from those that are outbred. Because of the large number of Mendelian factors concerned, as well as a large number of environmental factors, significant differences could only be expected if several hundred daughters of a given male are considered. The fact that no significant difference in winter egg yield comes from mating the same male to related females and unrelated females indicates that both groups of females were of about the same factor constitution for egg production.

Unfortunately, we are unable to check the inbred pullets hatched in 1920 and 1921 against outbred daughters of the same sire, because the males used in inbreeding were not mated to any unrelated hens. One obvious fact indicated by the table is the decline in winter egg production of both dams and daughters used during these two years. The fact is evident that the descendants inbred to hen B357 did not lay as many eggs as B357.

Winter rate of production is calculated by dividing the number of eggs laid during the pullet year before March by the number of days between the first and last egg. One very noticeable feature is the apparent decline in rate as inbreeding progressed. The outbred birds hatched in 1919 exhibit no significantly higher rate than their inbred sisters. During the second and third year of the experiment, however, the winter rate became so low that the mean egg production is very mediocre.

The length of winter pause did not increase with the degree of inbreeding and behaves in ordinary Mendelian fashion, as will be shown later. In general, the decreased winter egg production with the increased degree of inbreeding may be explained as due largely to decreased early maturity and decreased winter rate of production.

The percentage of inbreeding of the daughters is calculated by Wright's (1922) method and the theoretical degree of homozygosity is calculated for each inbred mating. The latter figures serve as an indicator of the still

large percentage of heterozygosity in rather closely inbred birds. Inbreeding has here operated to make homozygous some of the factors detrimental to high egg yield. Evidently the foundation hen, although a good individual as far as individual criteria may be recognized, carried factors which when intensified reduced the winter egg production as the experiment progressed.

The gross effects of inbreeding upon winter egg production may be studied through the daughters' coefficients of variability as given in the last column of Table I. The 1919 hatch shows a rather wide coefficient of variability as might be expected. The smaller range in variability of outbred daughters compared with inbred daughters is contrary to expectation and evidently due to experimental error entirely. The very large coefficients of variability that exist when full sisters are considered has caused many to question the inheritance of egg production according to Mendelian laws. Coefficients of variability are consistently smaller for the 1921 daughters, even though the number from each dam is small and more males were used than in 1919, so that the results are subject to larger error. The general assumption may be drawn from these very limited results that variability in winter egg yield may be reduced by breeding methods.

Effect of Inbreeding on Weight at First Egg

Body weight is often considered an index of vigor. In Table II are presented the weights of each dam at her first egg, together with the average weight of all her daughters at first egg.

Table II clearly shows that the weight at first egg was amply maintained regardless of inbreeding. The second and third year inbreds were older when the weight at first egg was recorded and this would give them an advantage.

Fertility and Hatchability

Fertility and hatchability records are available on only a limited number of the hens included in this report.

TABLE II

WEIGHT OF PULLETS AT FIRST EGG

1919 Hatch	*Lbs.*	*Oz.*	*1920 Hatch*	*Lbs.*	*Oz.*
B357	4	10	B357	4	10
2 Daughters	5	8	5 Daughters	5	0
B359	5	9	B3512	6	8
9 Daughters	5	15	1 Daughter	6	7
B754	7	0	*1921 Hatch*		
8 Daughters	5	14	B8318	5	2
B699	5	6	8 Daughters	5	1
6 Daughters	5	8	B9796	5	5
B710	5	0	2 Daughters	5	2
7 Daughters	5	2	C23	4	11
B785	5	4	4 Daughters	5	9
7 Daughters	5	1	B9651	4	8
B1357	4	10	2 Daughters	4	11
13 Daughters	4	13	B357	4	10
B2219	6	3	2 Daughters	4	6
6 Daughters	5	13			

Records are available for all hens used as breeders, but for few of the daughters of each hen. A very noticeable decline has been observed particularly in hatchability and to lesser extent in fertility as the inbreeding increased. Foundation hen B357 has a record as follows for her four breeding seasons:

HEN B357 HATCHED 1917

1918 mated to B115		1920 mated to B5806	
Per cent. fertile	73	Per cent. fertile	100
Per cent. fertile hatched	63	Per cent. fertile hatched	50
1919 mated to B3808		1921 mated to B9275	
Per cent. fertile	99	Per cent. fertile	96
Per cent. fertile hatched	49	Per cent. fertile hatched	67

This hen shows a rather low degree of hatchability coupled with a high degree of fertility. With such a foundation, low hatchability might be expected.

THE GENETIC BASIS OF WINTER EGG PRODUCTION

Goodale and Sanborn (1922) have already pointed out that the egg record of the Rhode Island Red hens in the Massachusetts station flock depends upon five main characteristics: maturity, rate, broodiness, persistency and winter pause. In the interpretation of the inbreeding results we have not included persistency and for winter pause we choose to use the term "partial molt" for the

reason that all pauses of more than a week's duration are accompanied by partial molt.

Early Maturity

Early maturity has been pointed out by Goodale and Sanborn (1922) in Rhode Island Reds and by Hurst (1921) in Leghorns and Wyandottes as having an important bearing on egg production. Further studies made by the writer at this station show a negative correlation between age at first egg and annual production during the pullet year of about 43 per cent. with a small probable error. In other words, early maturity is associated with heavy egg yields. Just what the relationship of early maturity is to rate and broodiness has not yet been worked out. Evidence seems to point to an association between extreme early maturity and the occurrence of the partial winter molt, but there is no conclusive evidence on this point.

Results tabulated in this report as well as many more extensive records on the Massachusetts station Rhode Island Red flock seem to indicate that two dominant factors for early maturity are concerned. We call these E and E′. Either combined or alone, simplex or duplex, these factors produce a bird that normally begins laying at 215 days or less. Those birds that lack both factors E and E′ (ee′ birds) do not begin laying until 216 days or older. The factor E is sex-linked and factor E′ is not sex-linked and is independent. One factor is known to be sex-linked because early maturing hens sometimes have entire families of late daughters.

The age at which a pullet lays her first egg, without doubt, depends on a large number of environmental factors. Such factors operate to make different pullets of the same genetic composition for early maturity begin laying at from 150 to 215 days and those lacking both of these factors at from 216 to 300 days or more. A cumulative effect of the factors E and E′ has not been demonstrated, but may exist.

The results of the first year of inbreeding are summarized in Table III. Inbred pullets hatched during 1920 and 1921 appear to be abnormal in early maturity and are omitted. These birds seem to be depleted in what might be called "sex vigor." In other words, they fail to begin laying until two or three months older than normal. The general behavior may be illustrated by the age at maturity of the daughters of hen B357 on four successive years. Here the degree of inbreeding advances from 0 in the first year to 43.75 per cent. in the fourth year.

RELATION OF DEGREE OF INBREEDING TO AGE AT FIRST EGG

Year	*No.*	*Daughters Average Age*
1918	5	179 days
1919	2	199 "
1920	6	235 "
1921	2	189 "

In Table III are the individual hens mated in 1919 to male B3808 with the genetic formula of each for factors governing early maturity. Opposite each hen is her daughters' classification, together with the probable error of ratio. In only two cases, namely, the daughters of B785 and B1357, is the actual deviation of the ratio greater than the probable error of the expected ratio calculated according to Weldon (1902). Probably hen B785 was an early maturing hen but may have been held back by environment. In the case of hen B1357, only 13 daughters went through the winter normally and are included in this report. Her other nine daughters were abnormal and are not included because they never laid or laid only a few eggs in the fall or developed ovarian disorders and became "nesters." Some of these daughters were late maturing, so that the formula Eo E′e′ is thought to be correct.

The agreement of results with theory is close and is mathematically significant. The evidence of two dominant factors for early maturity seems rather conclusive even though the number of birds concerned in this report is small.

TABLE III

EARLY MATURITY

1919 Hatch

Up to 215 days = early
216 days or more = late

Sire B3808 (Ee E′e′)

Dam	Genetic Formula	Daughters Actual	Expected	
B 357	Eo E′e′	2	1.75 ± .31	early
		0	.25	late
B 359	Eo E′e′	8	7.875 ± .67	early
		1	1.125	late
B 754	eo e′e′	6	6.00 ± .82	early
		2	2.00	late
B 699	Eo E′E′	5	5.00	early
		0	0	late
B 710	Eo E′e′	6	6.125 ± .59	early
		1	.875	late
B 785	eo e′e′	7	5.25 ± .77	early
		0	1.75	late
B1357	Eo E′e′	13	11.375 ± .80	early
		0	1.625	late
B2219	eo e′e′	5	5.25 ± .77	early
		2	1.75	late

WINTER MOLT

The winter molt as referred to in this report probably corresponds with winter pauses of considerable length referred to by others. With the Rhode Island Reds there appears to be a close association between pauses of one week or more by pullets in the fall or winter and a partial molt. This fact has been checked up on large numbers. Furthermore, this tendency appears to be inherited on a single factor basis. By using only non-molt breeding females this winter loss of eggs has been greatly reduced. A study of its behavior in male breeders has not yet been made. There may also be linkage between factors for early maturity and molt. The factor M is used here to represent the gene for winter molt.

In the unimproved state, the hen generally begins laying in March or April and continues more or less irregularly for about three months. She then prepares herself for complete molt. By methods of breeding, selecting for extreme early maturity, housing, etc., most of the pullets now begin laying in the fall. If they still exhibit the natural tendency to molt in from two to three months after beginning to lay, they are spoken of as showing winter

molt. Severe weather conditions of winter seem in a measure to check this loss of feathers, so that only a partial molt is observed. The general belief is that molt is associated with a depleted metabolic condition and this may be brought about by the heavy strain of egg laying. Pullets that show no winter molt even though they begin to lay early, evidently differ genetically from the original type in regard to molt.

TABLE IV
WINTER MOLT

Dam	Genetic Formula	Daughters Actual	Expected	
1919 Hatch				
		Sire B3808 (Mm)		
B 357	mm	1	1	molt
		1	1	non-molt
B 359	Mm	7	6.75 ± .88	molt
		2	2.25	non-molt
B 754	Mm	6	6 ± .82	molt
		2	2	non-molt
B 699	mm	4	2.5 ± .76	molt
		1	2.5	non-molt
B 710	Mm	5	5.25 ± .77	molt
		2	1.75	non-molt
B 785	mm	3	3.5 ± .89	molt
		4	3.5	non-molt
B1357	mm	7	6.5 ± 1.21	molt
		6	6.5	non-molt
B2219	mm	4	3.5 ± .89	molt
		3	3.5	non-molt
1920 Hatch				
		Sire B5806 (Mm)		
B 357	mm	2	2.5 ± .76	molt
		3	2.5	non-molt
B3512	Mm	1	.75	molt
		0	.25	non-molt
1921 Hatch				
		Male B8273 (Mm)		
B8318	mm	2	2 ± .67	molt
		2	2	non-molt
B9796	mm	1	.5	molt
		0	.5	non-molt
C23	Mm	4	3 ± .58	molt
		0	1	non-molt
		Male B8574 (Mm)		
B8318	mm	1	1	molt
		1	1	non-molt
B9651	mm	0	1	molt
		2	1	non-molt
B9796	mm	0	.5	molt
		1	.5	non-molt
		Male B9275 (mm)		
B 357	mm	0	0	molt
		2	2	non-molt

There is a very close agreement between theory and result in Table IV. There is not a single instance in a family of sufficient size to calculate the probable error of expected ratio with any degree of accuracy, where the results deviate beyond the limits of probable error. There seems little ground for questioning the heritability of winter pauses of a week or more in duration.

Broodiness

Hens that go broody spend considerable time not laying during such periods. Broody periods may vary in length from a few days to several weeks. Goodale (1920) found no significant correlation between the number of days spent in broodiness and the annual egg production. The more frequent occurrence of broodiness during spring and summer months tends to reduce the importance of broodiness from the winter production standpoint. In this connection, the fact should be pointed out that, in the station flock of Rhode Island Reds, rate of production has been higher in the strains carrying a large percentage of broodiness than in the nearly broody-free strains. There is no evidence to indicate that there is any linkage between broody factors and rate factors, however. Combinations of high rate and non-broodiness appear to exist in the present flock.

The two-factor (A C theory) suggested by Goodale (1920) has been found to be substantiated by the inbreeding experiment. In brief, this theory assumes two dominant factors A and C for broodiness. Their combined action is necessary to produce broodiness. Males and females may carry either factor alone and only from those matings that bring together both factors will broody hens result.

Table V presented below gives the genetic composition of each breeding male and female together with the actual and expected proportion of broodies and non-broodies.

Table V indicates that the results fit expectations very well. Those families hatched during 1919 in which the

TABLE V

BROODINESS

Dam	Genetic Formula	Daughters Actual	Expected	
1919 Hatch			Sire B3808 (Aa Cc)	
B 357	Aa cc	0	.75 ± .45	Broody
		2	1.25	Nonbroody
B 359	Aa Cc (Broody)	4	4.5 ± .94	Broody
		4	3.5	Nonbroody
B 754	Aa cc	2	3.0 ± .92	Broody
		6	5	Nonbroody
B 699	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B 710	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B 785	Aa cc	2	1.86 ± .71	Broody
		3	3.14	Nonbroody
B1357	Aa cc	6	4.875 ± 1.17	Broody
		7	8.125	Nonbroody
B2219	aa cc	0	1 ± .59	Broody
		4	3	Nonbroody
1920 Hatch			Sire B5806 (aa Cc)	
B 357	Aa cc	0	1.25 ± .65	Broody
		5	3.75	Nonbroody
B3512	Unknown	No daughters		
1921 Hatch			Sire B8273 (aa Cc)	
B8318	Aa cc	1	1 ± .59	Broody
		3	3	Nonbroody
B9796	Aa cc	0	.25 ± .29	Broody
		1	.75	Nonbroody
C23	aa CC	0	0	Broody
	aa Cc	3	3	Nonbroody
	aa cc			
			Sire B8574 (aa Cc)	
B8318	Aa cc	1	1.25 ± .65	Broody
		4	3.75	Nonbroody
B9651	AA cc	1	1 ± .67	Broody
		1	1	Nonbroody
B9796	Aa cc	0	.5 ± .41	Broody
		2	1.5	Nonbroody
			Sire B9275 (Aa Cc)	
B 357	Aa cc	1	.75 ± .46	Broody
		1	1.25	Nonbroody

number of broodies is less than expected may be explained by the fact that they were only carried until May. Some of the later maturing pullets would probably become broody later. In no case where the number of pullets tested amounts to six or more is there a deviation

beyond the probable error. Further work is being done on the question to test the validity of the theory.

Winter Rate of Egg Laying

Rate as used in this report is calculated for each hen by dividing the number of eggs laid before March first of the pullet year by the number of days covered. High rate means 50 per cent. or more, or an average of at least one egg in two days during the winter season. Low rate is a percentage below fifty.

Evidence seems to indicate that two independent dominant factors R and R′ are concerned in high rate. The presence of both is necessary to produce a rate of 50 per cent. or more. Either factor alone gives less than 50 per cent. rate. These two factors seem to be concerned with rate in the same way that factors A and C are concerned with broodiness.

TABLE VI

Winter Rate of Production

1919 Hatch

Dam	Genetic Formula	Daughters Actual	Expected		
			Sire B3808 (Rr R′r′)		
B 357	RR R′r′	2	1.5 ± .41		High
		0	.5		Low
B 359	Rr R′r′	4	4.5 ± .94		High
		4	3.5		Low
B 754	Rr R′r′	5	4.5 ± .94		High
		3	3.5		Low
B 699	Rr R′r′	2	2.8 ± .74		High
		3	2.2		Low
B 710	RR R′r′	6	4.25 ± .77		High
		1	1.75		Low
B 785	RR R′R′	7	7		High
		0	0		Low
B1357	Rr R′r′	7	7.3 ± 1.20		High
		6	5.7		Low
B2219	RR R′r′	6	4.25 ± .77		High
		1	1.75		Low

1920 Hatch

			Sire B5806 (Rr r′r′)		
B 357	RR R′r′	3	2.5 ± .75		High
		2	2.5		Low
B3512	Unknown	No daughters' records			

TABLE VI—*Continued*

1921 Hatch

			Sire B8273 (Rr R′r′)	
B8318	Rr R′r′	2	2.25 ± .66	High
		2	1.75	Low
B9796	Rr R′r′	0	.56 ± .33	High
		1	.44	Low
C23	rr r′r′	0	1 ± .58	High
		4	3	Low
			Sire B8574 (Rr R′r′)	
B8318	Rr R′r′	1	1.7 ± .57	High
		2	1.3	Low
B9651	RR R′R′	2	2	High
		0	0	Low
B9796	Rr R′r′	1	.56	High
		0	.44	Low
			Sire B9275 (RR R′R′)	
B 357	RR R′r′	2	2	High
		0	0	Low

Table VI gives the probable formula of each breeding hen and sire, also the actual and expected grouping of the daughters from each mating.

The agreement between the actual and theoretical grouping of the daughters for rate is rather close. The foundation hen B 357 appears to have been homozygous for R factor but heterozygous for R′. The impossibility of knowing the genetic make-up of males or females without a breeding test is well illustrated by the occurrence of "low rate" individuals throughout the experiment. By proper matings it should be entirely possible to establish strains of fowls breeding true for high rate.

GENETIC FORMULAE FOR FOWLS

The probable genetic formulae for the fowls used as breeders in this experiment are given below:

1919 Hatch

Sires		*Dams*	
B3808	Ee E′e Mm Aa Cc Rr R′r′	B 357	Eo E′e′ mm Aa cc RR R′r′
		B 359	Eo E′e′ Mm Aa Cc Rr R′r′
		B 754	eo e′e′ Mm Aa cc Rr R′r′
		B 699	Eo E′E′ mm Aa cc Rr R′r′
		B 710	Eo E′e′ Mm Aa cc RR R′r′
		B 785	eo e′e′ mm Aa cc RR R′R′
		B1357	Eo E′e′ mm Aa cc Rr R′r′
		B2219	eo e′e′ mm aa cc RR R′r′

								1920 Hatch								
B5806	.....	.....	Mm	aa	Cc	Rr	r′r′	B 357	Eo	E′e′	mm	Aa	cc	RR	R′r′
								B3512	.....	.....	Mm	.....	.....	.....	.....
								1921 Hatch								
B8273	.....	.....	Mm	aa	Cc	Rr	R′r′	B8318	.....	.....	mm	Aa	cc	Rr	R′r′
								B9796	.....	.....	mm	Aa	cc	Rr	R′r′
								C23	.....	.....	Mm	.....	.....	rr	r′r′
B8574	.....	.....	Mm	aa	Cc	Rr	R′r′	B8318	.....	.....	mm	Aa	cc	Rr	R′r′
								B9651	.....	.....	mm	AA	cc	RR	R′R′
B9275	.....	.....	mm	Aa	Cc	RR	R′R′	B9796	.....	.....	mm	Aa	cc	Rr	R′r′
								B 357	Eo	E′e′	mm	Aa	cc	RR	R′r′

All blank spaces represent factors not determined by breeding tests. The above outline serves to show the complex of genetic factors concerned with winter egg production and makes clear the difficulty in establishing true-breeding flocks.

Summary

Inbreeding reduces variability in winter egg production only when the foundation stock is largely homozygous for factors for heavy egg yield.

Sexual maturity seems to be retarded in many inbred pullets so that they are very slow in beginning to lay.

Body weight does not appear to be affected by inbreeding.

Winter egg yield shows a tendency to decline after the degree of inbreeding passes 25 per cent., but not necessarily so. A cumulative effect may be observed in succeeding generations.

Winter egg production probably depends on seven pairs of Mendelian factors. The development of a flock breeding true for these factors is entirely possible by proper methods.

LITERATURE CITED

East, E. M. and D. F. Jones. Inbreeding and Outbreeding. Philadelphia, 1919.

Goodale, H. D. Mass. Agri. Experiment Station Bul. 199, 1920.

Goodale, H. D. and Ruby Sanborn. Mass. Agri. Exp. Sta. Bul. 211, 1922.

Hurst, C. C. *Nat. Poultry Jour.*, 1921.

Weldon, W. F. R. Mendel's Law of Alternative Inheritance in Peas. Biometrika 1. 1902.

Wright, Sewall. U. S. Dept. of Agr. Bul. 1121, 1922.

Wright, Sewall. Coefficients of Inbreeding and Relationship. AMER. NAT., 56, 1922.

THE DISTRIBUTION OF CHROMOSOMES IN TETRAPLOID DATURAS

JOHN BELLING AND ALBERT F. BLAKESLEE
STATION FOR EXPERIMENTAL EVOLUTION, COLD SPRING HARBOR[1]

THIS article is the second paper dealing, somewhat in detail, with the distribution of the chromosomes in *Daturas* with aberrant chromosome numbers, the first paper being that on triploids (3). A description of the microscopical methods used will be found in a short note in the AMERICAN NATURALIST (2).

Attraction of homologous chromosomes: At the late prophase and the metaphase of the first division in the pollen-mother-cells of true tetraploid *Daturas,* the chromosomes are, as a rule, arranged in connected sets of four each, the four chromosomes of each quadrivalent being of the same size (4). (Quadrivalents have been observed in tetraploid mosses by the Marchals (8)). The formula of such a tetraploid group is, $L_4 + 4l_4 + 3M_4 + 2m_4 + S_4 + s_4$, the letters representing the six size classes. A not uncommon arrangement is that of two rings connected at one side (lower right of Fig. 1, *l* and *m*), so as to form a figure of eight. In such quadrivalents, each chromosome is attached to one chromosome at one end, and to three chromosomes at the other end. Often, however, perhaps more often, the two rings are folded together (upper left of Fig. 1, *l* and *l*) so that each chromosome is attached to three others at each end. The homologous chromosomes may also form a single ring with two chromosomes attached at one side of it, so that one end of each of the two is free (*L* in Fig. 1). Several

[1] Paper read in abstract at the genetics section of the Botanical Society of America, December 27, 1922.

other arrangements are also found (such as *s* and *l* above the center of Fig. 1).[2]

Separation (disjunction) of chromosomes: The pulling apart of the quadrivalents is usually difficult to follow. In the observed cases of the double ring in one plane,

FIG. 1. Metaphase of the first division in a pollen-mother-cell of a tetraploid Datura. The preparation was fixed and stained in iron-acetocarmine. Subsequently the cytoplasm was pressed from the cell and adhered closely to the cover-glass. (This permitted the advantageous use of an apochromatic oil immersion objective of 1.4 aperture, with yellow-green light (2), and an immersed achromatic condenser of high aperture.)

The segmentation of each chromosome is visible. The four constituent chromosomes can be seen readily in eleven of the quadrivalents. There are 5 figures of eight (*l, M, M, m, m*), two double hoops (*l, l*), 2 examples of a ring with a V (*L, M*), one cross (*s*), one bent rod (*l*), and one quadrivalent (*S*) which is not readily classified.

two chromosomes went towards one pole and two towards the other, as was shown by the attachment of the fibers. The distributions of the chromosomes after the reduction division prove, however, that in some quadrivalents, three chromosomes must have passed to one pole and one to the other.

[2] Quadrivalents were not found by Gates (*Arch. f. Zellforsch.*, 1909), or by Davis (*Ann. Bot.*, 1911), in tetraploid Oenothera Lamarckiana. They are difficult to demonstrate in the tetraploid Primula sinensis; but if several hundred well-fixed first metaphases are examined, and compared with those of the diploid form, it seems as if the majority of the 48 chromosomes were usually arranged in the tetraploid Primula in sets of two pairs each, and rarely 12 such sets may be counted.

Non-disjunction, single, double and triple: The chromosomes were counted after the reduction division, in the second metaphase, in more than 1500 pollen-mother-cells from true tetraploids. The first counts of the chromosomes of 24 tetraploid plants from different lines gave, in 218 pollen-mother-cells, 68 per cent. of $24 + 24$ distribution (Fig. 2), 30 per cent. of $23 + 25$ and 2 per cent. of $22 + 26$, with one case of $21 + 27$. This shows that non-

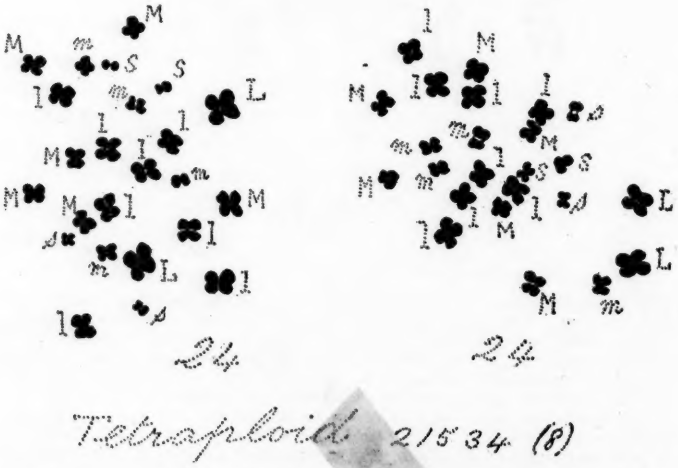


FIG. 2. Metaphases for the second division in the pollen-mother-cell. The preparation was made in the same way as that from which Fig. 1 was drawn with the camera. The sizes of the chromosomes are distinct whenever they are pressed into horizontality. The extra large (*L*), the small (*S* and *s*) and the small medium (*m*) are most easily distinguishable. The distinctions between *l* and *M*, and between *S* and *s*, are less easily made in this preparation.

disjunction is a regular phenomenon in tetraploid *Daturas*. (Gates observed a similar occurrence in tetraploid *Oenotheras* (6)). In order to study this further, one tetraploid plant was selfed, and the progeny (63 plants) grown to maturity. The chromosomes were counted in about twenty pollen-mother-cells, more or less, in all but one of these plants. The proportions of plants with different chromosome numbers are given in Table I.

TABLE I

NUMBERS OF CHROMOSOMES IN EACH OF 62 PLANTS OF THE SIBSHIP 21404

Numbers of chromosomes	48	49	47	(48)	50	46
Number of plants—						
27 (first lot)	25	2	.....	.....	.....	.....
35 (second lot)	30	3	1	1(?)	.....	.....
Totals	55	5	1	1	.....	.....
Calculated: (1) if only 2n pollen is functional	47	8	8	.....	.....	.....
(2) if 2n, (2n + 1), and (2n — 1) pollen functions	35	12	12	2	1	1

(In Table I, the 22-chromosome, or 26-chromosome, egg-cells are neglected in the calculation.)

The first lot in this table contains those seedlings potted out first from the seedpan; the second lot those of somewhat slower germination. There is an obvious advantage in having two groups to compare, even if no marked differences are noticeable.

If the chromosomes of the megaspores are distributed as are those of the pollen, then either the 24-chromosome egg-cells, or the 4n zygotes must evidently be more viable than the others. This has been found to happen in a (4n + 1) *Datura* investigated with regard to this point. (Van Overeem (9) has obtained somewhat similar results with the progeny of a tetraploid *Oenothera*).

The "(48)" plants in the table are pseudo-tetraploids, with 48 chromosomes, but with one set of three and one set of five.

All 48-chromosome plants in the sibship, having 20 or more than 20 pollen-mother-cells counted, are classified in Table II.[3]

With one exception, the deviations from 25 per cent. do not seem too great to be due to random sampling,

TABLE II

CLASSIFICATION OF 37 48-CHROMOSOME PLANTS FROM THE SIBSHIP 21404, ACCORDING TO THEIR PERCENTAGES OF THE 23 + 25 DISTRIBUTION OF CHROMOSOMES

Percentage	0	5	10	15	20	25	30	35	40	45	50	55	60
No. of plants	1	3	.....	4	6	10	5	4	3	.....	.....	1	

[3] The majority of the countings in sibship 21404 were done by Miss A. D. Bergner and Miss E. M. Lord.

since only a few more than 20 pollen-mother-cells were counted on the average in each plant. However, the plant with 59 per cent. of 23 + 25 distribution has less than one chance in a thousand for its random occurrence. It is probably a pseudo-tetraploid and is therefore omitted from Table III.

TABLE III

DISTRIBUTION OF CHROMOSOMES IN 1379 POLLEN-MOTHER-CELLS OF THE 55 TRUE TETRAPLOID DATURAS IN SIBSHIP NO. 21404

No. of plants	Chromosome groups	Distribution of chromosomes (Percentages)			Total No. of pollen-mother-cells
		24 + 24	23 + 25	22 + 26	
25	Single	75.5	21.8	2.7	404
30	Single	66.2	31.6	2.2	506
25	Double	73.6	24.7	1.8	284
30	Double	73.1	24.2	2.7	182

In the 1379 pollen-mother-cells in Table III there were two cases of 21 + 27 chromosomes, and one of 20 + 28. (The 22 cases of detachment (elimination) found, could not, of course, be included here; but will be discussed later.)

In Table III the distributions of chromosomes in the double groups from the two sets of early and late plants resemble one another closely, and are doubtless somewhat more reliable than the distributions resulting from the single groups. Hence, we may take the total of all the double groups in the true tetraploid plants of the sibship 21404 as forming the most reliable datum. This is 342 (73.2 per cent.) of 24 + 24, 114 (24.4 per cent.) of 23 + 25, 10 (2.1 per cent.) of 22 + 26 and 1 (0.2 per cent.) of 21 + 27.

If non-disjunction occurs with the same frequency in each quadrivalent, there would be a certain number of cases of double non-disjunction, both non-disjunctions being on the same side, thus giving a distribution of 22 + 26 chromosomes. There would also be an equal number of cases of double non-disjunction on opposite sides (that is, double opposed non-disjunction), giving a distribution of 24 + 24 chromosomes; each group con-

taining one set of one, and one set of three chromosomes, instead of two chromosomes to each set. Triple non-disjunction would give either 21 + 27, or 23 + 25; the latter distribution being three times as numerous as the former. Quadruple non-disjunction is probably negligible. Hence, if x : 1 is the ratio of ordinary disjunction to non-disjunction in any quadrivalent (neglecting double non-disjunction in the same quadrivalent), the distribution of non-disjunction would, of course, be proportional to the terms of the binomial $(x + 1)^{12}$. Only the first four terms are important here. Reduced, they are x^3, $12x^2$, 66x, 220. Adding to the first term the 33x cases of double opposed non-disjunction (which give the distribution 24 + 24), and adding to the second term the 165 cases of triple opposed non-disjunction (which give 23 + 25), we have the proportion in column 3, Table IV.

TABLE IV

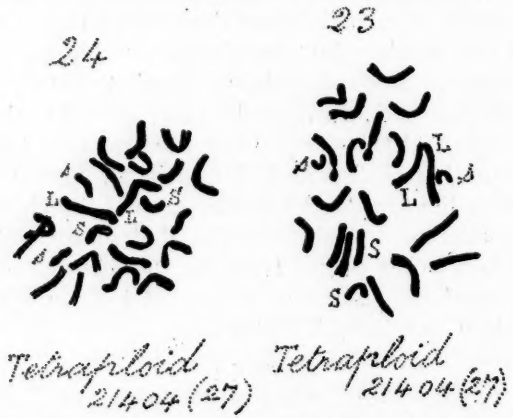
CALCULATION OF CHROMOSOME DISTRIBUTION

Chromosome Distribution	Numbers Found	Theoretical Proportions	*Numbers Calculated*
24 + 24	342	$x^3 + 33x$	(342)
23 + 25	114	$12x^2 + 165$	115.5
22 + 26	10	33x	9.0
21 + 27	1	55	0.4
	Total 467		Total 466.9

The third column in Table IV gives the theoretical proportions corresponding to the assumption that non-disjunction is the same in all 12 quadrivalents. By trial we get approximately 35 as the value of x, taking the first number as 342 and the total as 467. We then obtain for the second number, 115.5, for the third, 9.0, and for the fourth 0.4. From the fairly close agreement of the third and fourth numbers especially, we may assume that non-disjunction possibly takes place in each quadrivalent in about one thirty-sixth, or nearly three per cent. of the cases of possible disjunction.

From observation of the distribution of chromosomes after the reduction division in the pollen-mother-cells, we assume that about one quarter of the pollen-grains have 23 or 25 chromosomes. To test this, pollen-grains at the

stage of the first nuclear division were fixed and stained from a true tetraploid plant, No. 21404 (27). This plant had 25 per cent. of the 23 + 25 distribution. Fifteen pollen-grains gave certain counts (Figs. 3 and 4).



FIGS. 3 and 4. Metaphases of the first division in the pollen-grain. The young pollen-grains were fixed and stained as before. The cells were somewhat flattened by the capillary pressure of the cover-glass. The line of the longitudinal division is distinct at the metaphase in the pollen-grains examined. This metaphase is obviously easier to count than the corresponding stage in the root-tips; but the second metaphase of the pollen-mother-cell is superior to both for counting, because of the shortness of the chromosomes.

TABLE V

CHROMOSOME DISTRIBUTION IN THE POLLEN-GRAINS

No. of chromosomes	24	23	25
No. of pollen-grains	11	3	1
Calculated	11.2	1.9	1.9

The numbers in Table V, though few, confirm the conclusions from the chromosome counts at the second metaphase in the pollen-mother-cells.

Detachment (elimination) of chromosomes: After the reduction division, one or two (rarely more) chromosomes are sometimes left detached between the two nuclei. These chromosomes often divide at the second division and form minute cells, microcytes, which perish (3). In the total of 1,401 pollen-mother-cells examined from the 55 tetraploid Daturas of the sibship 21404, there were

TABLE VI

DETACHMENT (ELIMINATION) OF CHROMOSOMES. NON-REDUCTION

Pollen tetrads of tetraploid plants. (Percentages)

Microspores / Microcytes		4	4 1	4 2	4 3	4 4	2	Etc.	Percentage of detachment	No. of tetrads
Plant, and bud	Percentage of 23 + 25 distribution									
20567(1)	27	99.8	.....	.....	.....	.....	0.2	.....	0.0	401
21107(1)	26	98.4	0.6	0.9	.....	.....	.....	0.1	1.6	1002
21181(10)	27	96.7	1.0	2.2	.....	0.1	.....	.....	3.3	827
21404(6)	18	93.8	4.6	1.7	.....	.....	.....	.....	6.3	416
21404(71)	25	98.0	0.5	1.2	.....	.....	0.3	0.1	1.8	1225
21404(80)a	27	98.2	0.9	0.9	.....	.....	0.1	.....	1.7	940
21404(80)b	27	97.4	1.3	1.2	.....	.....	.....	.....	2.6	821
21536(8)	21	96.4	1.0	1.7	.....	.....	0.8	0.1	2.8	830
Percentages of totals		97.5	1.0	1.3	.....	0.02	0.2	0.05	2.3	6462 (total)

22 observed cases of detachment of one or more chromosomes at the reduction division, which is 1.6 per cent. The total amount of detachment can be reckoned from the percentage of pollen tetrads which show microcytes (which are small cells containing the detached chromosomes). Table VI gives the numbers of microcytes seen in over 6,000 pollen tetrads from 7 true tetraploid plants. They show one rather excessive amount of detachment, namely, 6.3, and there is perhaps reason to suppose some effect of environment, as in triploids (3). The total percentage of detachment for the 6,462 tetrads is 2.3, which may not be significantly different from the 1.6 per cent. of detachment at the first division obtained by direct observation in 1,401 pollen-mother-cells. Any detachment at the second division may therefore be infrequent, as is also shown in triploids (3).

Non-reduction: The omission of the reduction division is rare in the tetraploids examined. A 48 + 48 chromosome distribution results. The longitudinal division of each of the full number of chromosomes in the first metaphase plate without reduction has been observed more frequently in modified tetraploids with one or two extra chromosomes. The results of non-reduction, namely, two giant cells from each pollen-mother-cell, and giant pollen-

grains of double the ordinary volume, have also been observed in true tetraploids. Table VI shows that double-sized microspores were formed from about 0.2 per cent. of the pollen-mother-cells. There would be produced about half of this percentage of giant pollen-grains. From each of three plants which were apparently true tetraploids, 100 pollen-grains were measured. Out of these 300 grains, one alone had twice the average volume.

Chromosomes of functional egg-cells: Tetraploid Daturas were pollinated with pollen of diploids. The results are given in Table VII.

TABLE VII

CHROMOSOMES OF PROGENY OF TETRAPLOID DATURAS POLLINATED BY DIPLOID

Numbers of chromosomes	2n	2n + 1	3n − 1	3n	3n + 1	4n
Numbers of plants	14	2	1	7	1	1
Calculated	....	....	1.1	6.8	1.1	....

The mode of origin of the $2n$ and $(2n + 1)$ plants in Table VII is unknown at present, but experiments to ascertain it are being undertaken. (The presence of one $4n$ plant may perhaps have been due to accidental selfing.) The remaining plants of the progeny apparently show that the megaspores also may have about 25 per cent. of the $23 + 25$ distribution of chromosomes after the reduction division, since a calculation on this basis fits closely to the facts.

Tetraploid (tetrasomic) inheritance: It may be shown (5) that the amount of non-disjunction occurring in a true tetraploid is not sufficient to cause a marked change in the Mendelian ratios for any particular pair of allelomorphs in the immediate offspring of a heterozygote. Since the general Mendelian results agree with expectation on the hypothesis of random assortment of the 4 chromosomes of a quadrivalent (7, 5); it follows, since each quadrivalent usually consists of two connected pairs, that the coming together of the members of these pairs must have been at random. The change of a true tetraploid to a double diploid would result from the two chromosomes of each of the two pairs having a preferential attraction.

Summary: (1) The chromosomes at the late prophase and metaphase of the first, or reduction division, in true tetraploid Daturas, are as a rule joined in quadrivalents, which in most cases are formed of two connected pairs.

(2) In each of the 79 true tetraploid plants examined there was a certain amount of the $23 + 25$ chromosome distribution after the reduction division. This averaged in the most reliable lot of counts, namely, the double groups from 55 sibs, nearly 25 per cent.

(3) The distribution of the chromosomes after the reduction division in true tetraploids, in the 467 cells with double groups, agreed closely with the distribution calculated for 35 cases of $2 + 2$ disjunction to 1 case of $3 + 1$ non-disjunction in the quadrivalents.

(4) Detachment (elimination) of chromosomes occurred in 2.3 per cent. of the pollen-mother-cells, chiefly (or wholly) at the reduction division. Non-reduction happened in 0.2 per cent. of pollen-mother-cells.

(5) On pollinating tetraploids by diploids, the proportions of $3n$, $(3n - 1)$, and $(3n + 1)$ progeny agreed with the hypothesis that there was approximately 25 per cent. of the $23 + 25$ distribution of chromosomes in the reduction division of the megaspore-mother-cells.

(6) When tetraploid Daturas are pollinated by diploids, there are produced also many diploid progeny, whose mode of origin is as yet unknown.

LITERATURE CITED

(1) Belling, J.
1921. The Behavior of Homologous Chromosomes in a Triploid Canna. *Proc. Nat. Acad. of Sciences*, 7: 197–201.

(2) Belling, J.
1923. Microscopical Methods Used in Examining Chromosomes in Iron-acetocarmine. AMER. NAT., 57: 92–96.

(3) Belling, J. and A. F. Blakeslee.
1922. The Assortment of Chromosomes in Triploid Daturas. AMER. NAT., 56: 339–346.

(4) Belling, J. and A. F. Blakeslee.
1923. The Reduction Division in Haploid, Diploid, Triploid and Tetraploid Daturas. *Proc. Nat. Acad. of Sciences.* (*In press.*)

(5) Blakeslee, A. F., J. Belling and M. E. Farnham.
1923. Inheritance in Tetraploid Daturas. *Bot. Gaz.* (*In press.*)
(6) Gates, R. R.
1911. Pollen Formation in Oenothera gigas. *Ann. Bot.*, 25: 909–940.
(7) Gregory, R. P.
1915. On the Genetics of Tetraploid Plants in Primula sinensis. *Proc. Roy. Soc.* (*B.*), 87: 484–492.
(8) Marchal, Él. et Ém.
1911. Aposporie et sexualité chez les Mousses. *Bul. de l'Acad. roy de Belgique* (*Classe des sciences*), 9–10: 750–778.
(9) van Overeem, C.
1922. Über Formen mit abweichender Chromosomenzahl bei Oenothera. *Beih. z. Bot. Centralbl.*, 39(1): 1–80.

EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. IX. NEW LIFE TABLES FOR DROSOPHILA[1]

RAYMOND PEARL AND SYLVIA L. PARKER

In the first of these Studies (27) we presented life tables for wild type and quintuple flies. These tables were based upon the collected data then available from the control portions of experiments in which these two sorts had been used. In the period which has elapsed since the publication of these pioneer life tables for *Drosophila* our work has been greatly extended, and in a number of particulars refined. Especially we have come to use in all the experimental work stocks which are more homogeneous *genetically.* Thus, for a long-lived stock we now use, instead of a random sample of a mass culture of wild type flies as was formerly the case, a random sample of our line 107, which is an inbred, long-lived "pure" strain. The origin of this line we have described in the second of these Studies (32). By "pure" we mean, of course, only that it is a highly inbred and homozygous strain. Similarly, we have come to use for a short-lived stock in experimental work pure vestigial strains, the study of Gonzalez (62) having shown that it is this mutant gene alone which is chiefly responsible for (or invariably associated with) the observed brachybioty of quintuple flies.

It seems desirable now to present new life tables for these genetically more homogeneous groups, in order that they may be at hand for reference in connection with further studies shortly to be published. Furthermore, we have not hitherto published any *Drosophila* life tables with age reckoned on a "centile of the equivalent life

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 95.

span'' base, except in the case of wild type males from our original life tables.[2]

The actual observations on which the new life tables of this paper are based are presented in Table I. It will be seen that they include a total of 2,822 wild type flies of line 107, and 980 pure vestigial flies. The observations are recorded on a 6 day base unit in the case of wild type flies, and a 3 day base unit in the case of vestigials. We have found these units sufficiently fine for purposes of tabulation and derivative computation. The actual observations were made daily; the data as presented in Table I have been subsequently grouped.

TABLE I

OBSERVED DEATHS (d'_x) AND SURVIVORSHIP (l'_x) IN WILD TYPE LINE 107, AND PURE VESTIGIAL FLIES

Age in days	Wild type, Line 107 Males d'_x	Males l'_x	Females d'_x	Females l'_x	Age in days	Pure Vestigial Males d'_x	Males l'_x	Females d'_x	Females l'_x
1	18	1000	23	1000	1	1	1000	11	1000
7	12	987	16	984	4	48	998	43	979
13	26	979	34	972	7	81	893	43	897
19	85	960	86	941	10	86	715	57	815
25	99	900	86	888	13	81	526	55	706
31	132	829	96	827	16	51	349	63	601
37	187	736	159	759	19	33	237	47	481
43	173	603	152	647	22	35	164	51	391
49	206	480	162	539	25	22	88	27	294
55	285	333	275	425	28	5	39	35	242
61	82	131	156	230	31	4	29	30	176
67	62	72	99	120	34	3	20	17	118
73	36	28	64	50	37	3	13	14	86
79	3	3	6	5	40	2	7	14	59
85	1	1	1	1	43	1	2	4	32
—	—	—	—	—	46	—	—	6	25
—	—	—	—	—	49	—	—	4	13
—	—	—	—	—	52	—	—	2	6
—	—	—	—	—	55	—	—	1	2
Totals	1407	—	1415	—	Totals	456	—	524	—

In the graduation of the material the same plan was used as in the construction of the earlier life tables (*loc. cit.*).

The equations in the present case are as follows:

Wild Type, Line 107—Males;

$$\log l_x = e^{-.02252584x}(2.9999414 - .0674377x + .000677752x^2 - .00000369321x^3).$$

Wild Type, Line 107—Females;

$$\log l_x = e^{-.02841248x}(3.0000256 - .0761521x + .0000854457x^2 - .00000449705x^3).$$

Vestigial—Males;

$$\log l_x = e^{-.06729445x}(2.9961959 - .1959626x + .00456415x^2 - .0000377354x^3).$$

Vestigial—Females;

$$\log l_x = e^{-.03916697x}(3.0019283 - .1188025x + .00165311x^2 - .00000842816x^3).$$

[2] *Cf.* Pearl (61), and Pearl and Doering (63).

The observations and fitted lines are shown in Fig. 1.

As a whole the fits are reasonable, if one has as an objective simply the general sweep of the observations and is not concerned, as the actuary is, to represent every

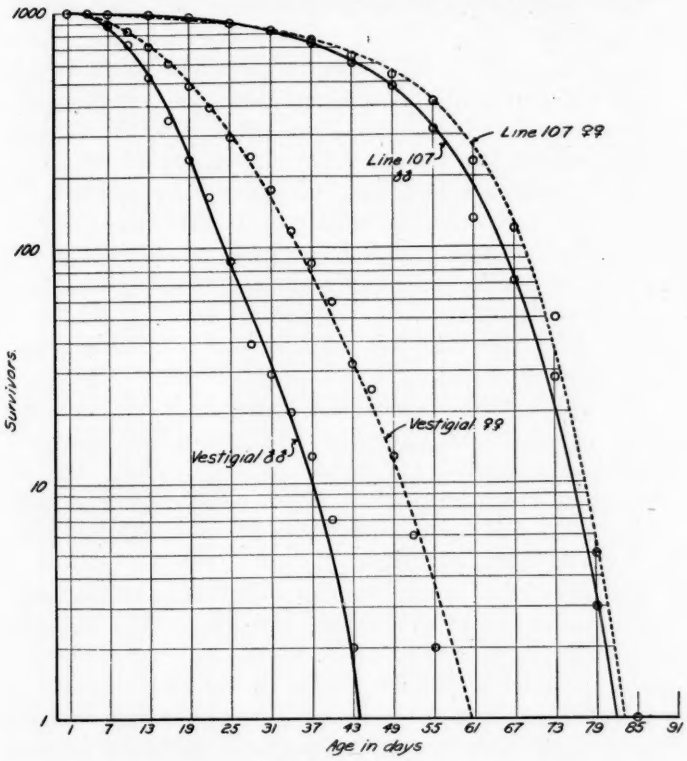


FIGURE 1

Diagram showing the observed and graduated l_x points for (a) line 107 wild type, and (b) vestigial flies. The small circles are the observations from Table I, and the smooth lines the fitted curves from the equations.

fluctuation in the curve. From this point of view the line 107 curves are entirely satisfactory. The vestigial curves are close fits up to 31 days in the females and 49 days in the males. The upper tails of both vestigial curves are bad fits, underestimating the observations in the females and overestimating in the males. To take

care of these end observations would require additional constants in the equations. But for all purposes to which fly life tables are ever likely to be put the present graduations will probably be adequate.

The complete life tables are presented in Tables II, III, IV and V.

From these tables the following points are to be noted:

(1) As compared with the genetically more heterogeneous earlier life tables, the purer strains of the present tables exhibit (a) a greater expectation of life at emergence in both sexes of line 107, but a shorter total life span than in the general wild type population; (b) substantially the same expectation of life at emergence and total life span, in male vestigials as in male quintuples; and (c) a distinctly longer expectation of life at emergence and longer total life span, in female vestigials than in female quintuples.

(2) These tables show the same relation between sexes in respect of mortality that human life tables do. The females have lower q_x values (deathrates) than do the males, throughout life. The sex differences in mortality are much more pronounced in the vestigials than in the wild type line 107.

(3) The form of the vestigial life curve is distinctly different from that of the wild type flies. The vestigial mortality is characterized by a plateau of nearly constant q_x values in middle life (in the males forming even a slight dip convex to the base). This phenomenon gives the vestigial l_x curves their peculiarly flattened appearance in the middle portion of their course.

It is desirable to compare these new *Drosophila* life tables with each other and with the human tables by putting the ages upon a relative base, using as a unit a centile (a hundredth part) of the equivalent life span, in the manner described by Pearl (61). The data of Tables II to V are transferred to a centile age basis in Table VI.

In Fig. 2 these centile distributions for *Drosophila* are compared with similar data from (a) human life tables (Glover (51)), and (b) the saturniid moth *Telea polyphe-*

TABLE II

LIFE TABLE FOR DROSOPHILA—WILD TYPE. LINE 107—MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.2	45.8	46	551	43.5	12.3
2	1000	0.6	44.8	47	527	46.6	11.8
3	999	1.0	43.8	48	502	49.8	11.3
4	998	1.3	42.9	49	477	53.2	10.9
5	997	1.7	41.9	50	452	56.9	10.4
6	995	2.0	41.0	51	426	60.8	10.0
7	993	2.4	40.1	52	400	65.0	9.6
8	991	2.8	39.2	53	374	69.5	9.2
9	988	3.2	38.3	54	348	74.2	8.8
10	985	3.5	37.4	55	322	79.2	8.4
11	981	3.9	36.5	56	297	84.5	8.0
12	978	4.3	35.7	57	272	90.2	7.7
13	973	4.7	34.8	58	247	96.2	7.4
14	969	5.1	34.0	59	223	102.5	7.0
15	964	5.5	33.2	60	200	109.2	6.7
16	958	6.0	32.3	61	179	116.3	6.4
17	953	6.4	31.5	62	158	123.8	6.1
18	947	6.9	30.7	63	138	131.7	5.9
19	940	7.4	29.9	64	120	139.9	5.6
20	933	7.9	29.2	65	103	148.8	5.3
21	926	8.5	28.4	66	88	157.9	5.1
22	918	9.0	27.6	67	74	167.6	4.9
23	910	9.6	26.9	68	62	177.7	4.7
24	901	10.3	26.1	69	51	188.3	4.4
25	892	10.9	25.4	70	41	199.4	4.2
26	882	11.7	24.6	71	33	211.0	4.1
27	872	12.4	23.9	72	26	223.1	3.9
28	861	13.3	23.2	73	20	235.8	3.7
29	849	14.1	22.5	74	15	248.9	3.5
30	837	15.1	21.8	75	12	262.6	3.4
31	825	16.1	21.1	76	9	276.8	3.2
32	811	17.2	20.5	77	6	291.5	3.1
33	798	18.3	19.8	78	4	306.7	2.9
34	783	19.5	19.1	79	3	322.5	2.8
35	768	20.8	18.5	80	2	338.7	2.6
36	752	22.3	17.9	81	1	355.5	2.4
37	735	23.8	17.3	82	1	372.7	2.2
38	717	25.4	16.7				
39	699	27.2	16.1				
40	680	29.1	15.5				
41	660	31.1	14.9				
42	640	33.3	14.4				
43	619	35.5	13.8				
44	597	38.0	13.3				
45	574	40.7	12.8				

TABLE III

LIFE TABLE FOR DROSOPHILA—WILD TYPE. LINE 107—FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.6	48.0	46	619	30.7	14.2
2	999	1.1	47.1	47	600	33.0	13.6
3	998	1.5	46.1	48	580	35.6	13.0
4	997	2.0	45.2	49	560	38.4	12.5
5	995	2.5	44.3	50	538	41.4	11.9
6	992	2.9	43.4	51	516	44.7	11.4
7	990	3.3	42.5	52	493	48.3	10.9
8	986	3.7	41.6	53	469	52.3	10.4
9	983	4.2	40.8	54	444	56.5	9.9
10	978	4.5	39.9	55	419	61.1	9.5
11	974	4.9	39.1	56	394	66.1	9.0
12	969	5.3	38.3	57	368	71.4	8.6
13	964	5.7	37.5	58	341	77.3	8.2
14	959	6.0	36.7	59	315	83.4	7.8
15	953	6.4	35.9	60	289	90.2	7.4
16	947	6.7	35.2	61	263	97.3	7.0
17	940	7.1	34.4	62	237	105.0	6.6
18	934	7.4	33.6	63	212	113.2	6.3
19	927	7.7	32.9	64	188	122.0	6.0
20	920	8.0	32.1	65	165	131.3	5.7
21	912	8.4	31.4	66	144	141.3	5.4
22	905	8.7	30.6	67	123	152.0	5.1
23	897	9.1	29.9	68	105	163.2	4.8
24	889	9.4	29.1	69	87	175.1	4.6
25	880	9.8	28.4	70	72	187.7	4.4
26	872	10.2	27.7	71	59	201.0	4.1
27	863	10.6	27.0	72	47	215.0	3.9
28	854	11.0	26.2	73	37	229.7	3.7
29	844	11.5	25.5	74	28	245.2	3.5
30	835	12.0	24.8	75	21	261.3	3.3
31	825	12.5	24.1	76	16	278.3	3.2
32	814	13.1	23.4	77	11	295.9	3.0
33	804	13.7	22.7	78	8	314.4	2.8
34	793	14.4	22.0	79	6	333.4	2.7
35	781	15.2	21.3	80	4	353.2	2.5
36	769	16.0	20.6	81	2	373.6	2.4
37	757	17.0	19.9	82	1	394.7	2.2
38	744	18.0	19.3	83	1	416.6	1.9
39	731	19.1	18.6				
40	717	20.3	17.9				
41	702	21.7	17.3				
42	687	23.1	16.6				
43	671	24.8	16.0				
44	655	26.6	15.4				
45	637	28.5	14.8				

TABLE IV

LIFE TABLE FOR DROSOPHILA—VESTIGIAL—MALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	0.0	14.1	26	72	162.7	5.8
2	1000	9.0	13.1	27	61	162.5	5.7
3	991	18.1	12.2	28	51	162.0	5.6
4	973	27.4	11.7	29	43	161.1	5.5
5	946	36.7	10.7	30	36	160.8	5.4
6	912	45.8	10.1	31	30	160.7	5.3
7	870	55.4	9.6	32	25	161.5	5.1
8	821	64.7	9.1	33	21	163.6	4.9
9	768	73.8	8.6	34	18	167.7	4.6
10	712	82.8	8.2	35	15	174.5	4.4
11	653	91.5	7.9	36	12	184.8	4.1
12	593	100.0	7.6	37	10	198.5	3.8
13	534	108.1	7.3	38	8	219.6	3.4
14	476	115.9	7.0	39	6	246.0	3.1
15	421	123.1	6.8	40	5	279.6	2.8
16	369	129.9	6.7	41	3	320.9	2.5
17	321	136.2	6.5	42	2	370.4	2.3
18	277	141.8	6.4	43	1	427.7	2.0
19	238	146.8	6.3	44	1	491.9	1.7
20	203	151.2	6.2				
21	172	154.8	6.1				
22	146	157.8	6.0				
23	123	160.0	6.0				
24	103	161.5	5.9				
25	86	162.4	5.9				

mus, on the basis of our calculations of an ungraduated life table from data as to the duration of life of this form in the adult stage given by the Raus (64).

From these data it appears that:

(1) The distribution of mortality in the different parts of the biologically equivalent life span is substantially *identical* quantitatively in an inbred strain of *Drosophila* (line 107) and in human beings of the present time. That is to say, if we take as our base line biological age, mortality is distributed along that base line in quantitatively the same manner in man and a particular inbred strain of wild type *Drosophila.* This does not, of course, in the least warrant the assertion that the forces determining rates of mortality in the two cases are identical. In detail they obviously are not. So far as is known, for example, the tubercle bacillus is not pathogenic to *Droso-*

TABLE V

LIFE TABLE FOR DROSOPHILA—VESTIGIAL—FEMALES

Age in days	l_x	q_x	e_x	Age in days	l_x	q_x	e_x
1	1000	7.7	19.8	36	88	124.9	7.1
2	992	11.0	19.0	37	77	127.6	6.9
3	981	14.3	18.2	38	67	130.3	6.8
4	967	17.7	17.4	39	58	133.1	6.7
5	950	21.2	16.7	40	50	135.8	6.5
6	930	24.7	16.1	41	44	138.6	6.4
7	907	28.2	15.5	42	38	141.4	6.3
8	882	31.7	14.9	43	32	144.3	6.1
9	854	35.3	14.3	44	28	147.3	6.0
10	824	38.8	13.8	45	24	150.4	5.9
11	792	42.4	13.3	46	20	153.6	5.7
12	758	46.0	12.9	47	17	157.0	5.6
13	723	49.6	12.5	48	14	160.7	5.4
14	687	53.2	12.1	49	12	164.7	5.3
15	651	56.8	11.7	50	10	169.0	5.1
16	614	60.4	11.3	51	8	173.6	5.0
17	577	64.0	11.0	52	7	178.7	4.8
18	540	67.6	10.7	53	6	184.3	4.7
19	503	71.1	10.4	54	5	190.5	4.5
20	467	74.7	10.1	55	4	197.3	4.3
21	432	78.1	9.8	56	3	204.9	4.1
22	399	81.6	9.6	57	2	213.3	3.9
23	366	85.0	9.3	58	2	222.6	3.7
24	335	88.4	9.1	59	1	232.9	3.5
25	305	91.7	8.9	60	1	244.3	3.2
26	277	95.0	8.7	61	1	256.8	2.9
27	251	98.2	8.5				
28	226	101.4	8.3				
29	203	104.5	8.1				
30	182	107.5	8.0				
31	163	110.5	7.8				
32	145	113.5	7.6				
33	128	116.4	7.5				
34	113	120.1	7.3				
35	100	122.2	7.2				

phila. The facts only mean that different in their *qualitative* details as are the lethal forces which attack the two organisms, man and a certain kind of *Drosophila,* they are alike in their *quantitative* relations to biological age.

(2) In another kind of *Drosophila,* differing so far as is known from the kind mentioned in the previous paragraph *only* in respect of one single second chromosome gene (and its somatic expression), the distribution of mortality in respect to biological age is widely *different*

TABLE VI

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA BY CENTILES OF LIFE SPAN

Centiles of Equivalent Life Span	Line 107		Vestigial		Centiles of Equivalent Life Span	Line 107		Vestigial	
	♂	♀	♂	♀		♂	♀	♂	♀
0	1000	1000	1000	1000	50	654	689	137	168
1	1000	1000	1000	996	51	637	676	127	157
2	999	999	1000	991	52	620	662	118	146
3	999	998	998	984	53	602	648	110	136
4	998	996	994	976	54	583	634	102	127
5	997	994	989	968	55	564	619	94	118
6	996	992	982	958	56	545	604	87	110
7	994	990	973	947	57	526	588	81	102
8	992	988	963	935	58	506	571	75	94
9	990	985	951	922	59	486	554	70	87
10	988	982	938	909	60	466	537	65	80
11	985	979	923	894	61	446	518	60	74
12	982	975	907	878	62	426	499	56	68
13	979	971	889	861	63	405	480	52	63
14	976	967	870	844	64	384	460	48	58
15	973	963	851	826	65	363	440	44	53
16	969	958	830	807	66	342	419	41	49
17	965	953	808	788	67	321	398	38	45
18	961	948	785	768	68	300	377	35	41
19	957	943	762	748	69	278	356	32	37
20	952	938	738	727	70	258	334	30	34
21	947	933	713	706	71	240	312	28	31
22	942	927	688	685	72	221	290	26	28
23	936	921	663	663	73	202	269	24	26
24	930	915	638	641	74	184	248	23	24
25	924	909	613	619	75	167	227	21	22
26	918	903	587	597	76	150	207	19	20
27	911	896	561	575	77	135	188	18	18
28	904	889	536	553	78	120	169	17	16
29	897	882	511	530	79	106	151	15	14
30	890	875	486	508	80	94	134	14	13
31	882	868	462	487	81	82	118	13	12
32	874	861	439	466	82	72	103	12	11
33	865	854	416	445	83	62	89	11	9.4
34	856	846	393	425	84	53	76	10	8.4
35	847	838	371	405	85	45	64	9.2	7.6
36	837	830	350	386	86	38	54	8.4	6.8
37	827	822	330	367	87	31	45	7.6	6.0
38	816	814	311	348	88	26	37	6.9	5.4
39	805	805	292	330	89	21	30	6.2	4.8
					90	17	24	5.5	4.2
40	794	796	274	312	91	14	19	4.8	3.7
41	782	787	257	295	92	11	15	4.2	3.2
42	769	777	240	278	93	8.3	11	3.7	2.8
43	756	767	225	263	94	6.4	8.3	3.2	2.4
44	743	757	210	248	95	4.8	6.1	2.7	2.1
45	730	747	196	233	96	3.6	4.5	2.3	1.8
46	716	736	182	219	97	2.6	3.2	1.9	1.6
47	701	725	170	206	98	2.0	2.2	1.6	1.4
48	686	713	159	193	99	1.4	1.4	1.3	1.2
49	670	701	148	180	100	1.0	1.0	1.0	1.0

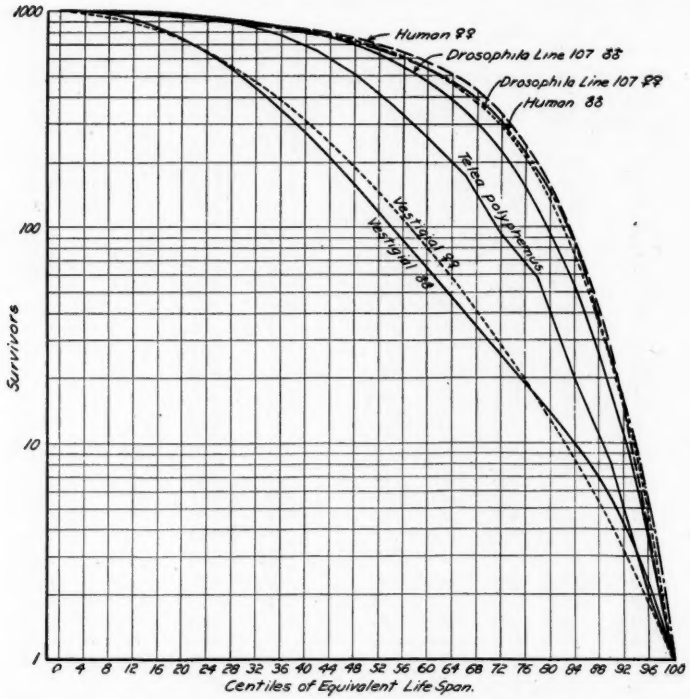


FIGURE 2

Comparing the survivorship distributions of (a) *Drosophila* line 107 males; (b) *Drosophila* line 107 females; (c) *Drosophila* vestigial males; (d) *Drosophila* females; (e) human males; (f) human females; (g) *Telea polyphemus*, both sexes together, over the equivalent life spans.

quantitatively from that found either in man or in wild type *Drosophila*, even in spite of the fact that both kinds of *Drosophila* spent their entire lives in statistically identical environments, so far at least as concerns temperature, optimum population density and housing, food, season and climate. This fact seems to us quite as significant as the identity established in the preceding paragraph. It shows that a unit change in the genetic constitution of an organism may not only be associated with a marked alteration of the absolute length of the life span, but also with a profound alteration of the form of the life curve.

(3) Wild type *Drosophila* and vestigial are plainly approximating two quite distinct theoretically possible forms of life curves. One of these types, which may be called the rectangular, would in the limit show all the individuals starting at birth together living to the same age and then all dying together at the same time. q_x would equal zero up to this "day of judgment," and then would on that day take the extreme value of 1,000 (on a per-thousand base). The closest approximation yet seen in living nature to the theoretical limit is *Proales,* as set forth by Pearl and Doering (63); the next closest approximation yet described is that of *Drosophila* line 107 and present day human beings, as shown in Fig. 2 above.

The other theoretical type of life curve which concerns the present discussion may be called the diagonal. This, in the limit, would be a case where the instantaneous death rate q_x would be constant at all ages from the start at birth to the demise of the last survivor. Plotted on arithlog paper the l_x line would be a straight diagonal line. The closest approach yet found in living nature to this theoretical type of life curve is that given by vestigial *Drosophila,* as shown in Fig. 2. All other life curves yet known fall between the rectangular and diagonal types.

There is a third type theoretically possible, but not actually realized in experience as yet. This is the case in which q_x has very large values in early ages, and thereafter nearly constant values until the last survivor is reached. This would mean an l_x line which dropped sharply to a low level in the earliest ages and then ran along a nearly horizontal course to the end of the life span of the last survivor. This would be the life curve of a very heavy selective mortality of early life. It is difficult to see how it could occur in a population genetically homogeneous in respect of factors influencing duration of life. But it could readily occur in a population genetically mixed relative to these factors.

LITERATURE CITED

(The plan of numbering citations is explained in the second of these Studies, AMER. Nat., Vol. 56, p. 174.)

62. Gonzalez, B. M. Experimental Studies on the Duration of Life. VIII. The Influence upon Duration of Life of Certain Mutant Genes of *Drosophila melanogaster*. AMER. NAT., Vol. 57, pp. 289–328. 1923.

63. Pearl, R., and Doering, C. R. A Comparison of the Mortality of Certain Lower Organisms with that of Man. *Science*, Vol. 57, pp. 209–212, 1923.

64. Rau, P., and Rau, N. Longevity in Saturniid Moths and its Relation to the Function of Reproduction. *Trans.* Acad. Sci. St. Louis, Vol. 23, pp. 1–78, pl. I–V, 1914.

BOOKS AND LITERATURE

A BIBLIOGRAPHY OF FISHES

DR. BASHFORD DEAN'S third and completing volume of "A Bibliography of Fishes," which has just been distributed by the American Museum of Natural History, will be considered with a feeling of great satisfaction by everyone who has an interest in any of the numerous and diverse subjects relating to fishes and fisheries.

The first two volumes of nearly 1,500 pages contain a bibliography arranged alphabetically by authors. It includes about 40,000 titles. Without the third volume the first two would be complete in themselves, but the use of them requires as a prerequisite a knowledge of author's names and subjects. Such knowledge the specialist has to a greater or less degree of completeness, though even in his own field memory often fails him. Consequently, the third volume is the most useful of them all, for its most important part is an index arranged under subject headings, and as it refers back to the other two volumes it serves as a key to them and makes a unit of the whole.

This invaluable work has been in preparation for thirty years. During this time Dr. Dean has had at least a dozen collaborators. The first two volumes were under the editorship of the late Dr. C. R. Eastman, who was succeeded by Dr. E. W. Gudger with the cooperation of Mr. A. W. Henn.

It might well serve as a model for a bibliography of each of the vertebrate classes. It is doubtful, however, if the bibliography of any other class would include so many titles, or so many names of illustrious authors, containing as it does such a large proportion of the greatest zoologists of all times.

Not only will the men interested in fishes be under great obligations to Dr. Dean and his colleagues, but comparative anatomists will be also, for the anatomy of the primitive vertebrates is fundamental to an understanding of all anatomy.

The literature relating to any one subdivision of zoology has grown so great in magnitude that no one of average memory can hope to keep in mind even that part of it relating to his own special field; and who does not more than occasionally wish to go afield?

The first caption of the subject index is *abdominal pores*, under which are many references, some of which are starred to indicate special importance. Besides this, there is a very adequate digest of the subject, including views of different authors

and probable homologies. This subject happens to be of present interest to the writer, for recently he wished to refer a student in comparative anatomy to the literature relating to abdominal pores in the Elasmobranchs and found his knowledge limited to the text-books. These, naturally having to cover the entire field of comparative anatomy, could not give more than the fair proportionate space of a few lines to this small part of it. This is cited as an instance of the immediate usefulness of the book. Scattered through the subject index are interpretations and notes of information.

Glancing hastily through the index the heading, *air-bladder*, catches the eye, with sub-heads covering development, homologies, anatomy, ducts, degeneration, specialization, gaseous content, functions and the conditions of the air-bladder in the different groups of fishes. In this way each part of anatomy is taken up, and each treated in the same exhaustive manner. The skeleton, for instance, is treated under its separate parts, as skull or pectoral girdle, and each followed through the separate fish groups. Under angling, general treatises are separated in different languages; history of angling is included; early laws, different methods of angling for different fishes and in different regions. Aquaria and aquarium fishes are very fully treated with long lists of papers on aquarium methods and on specific fishes.

Selecting a few of the headings at random one finds: color, behavior, commensalism, digestion, ecology, electric organs, functions, sex determination, faunas considered geographically, products and by-products, food of fishes and fishes as food, legislation and protection, preserving methods, the use of poison in capturing, reproduction and sound production. These are only a few of the diverse headings, but enough to show the scope of the work.

The subject index occupies only half of the third volume. This condensing is possible because the papers noted in the author's index are referred to by a scheme of abbreviations.

In the preface of the first and third volumes will be found a history of the work from its beginning. A very attractive feature lies in the quaint quotations from the old authors on the pages next to the title of the same volumes. In the selection of them we recognize the hand of our old friend, Dr. Dean. The writer may here take occasion to thank him and his collaborators for the bright light thrown on his chosen pathway by the bibliography of fishes.

EDWIN C. STARKS
STANFORD UNIVERSITY

SHORTER ARTICLES AND DISCUSSION

RADIUM RADIATIONS AND CROSSING OVER

DURING the summer of 1917 while at the Marine Biological Laboratory at Woods Hole, I made a series of preliminary tests of the effect of radium radiations on crossing over in the second chromosome of *Drosophila*. The radium used—50 mg. of the pure bromide—was very kindly loaned by Dr. Charles Packard, who was studying its effect on embryological processes.[1] Although there was clear evidence of a change in the percentage of crossing over, the results as a whole seemed contradictory, and publication of them was reserved until further tests could be made. There has been no opportunity to do this as yet, but the recent work of Mavor[2] and Mavor and Svenson[3] on the effect of X-rays on crossing over seems to place these data in a somewhat different light. For that reason a brief account of the results appears to be advisable.

The methods were similar to those used in my tests of the effect of temperature on crossing over[4] and followed more recently by Mavor. Five newly hatched females heterozygous for the second chromosome genes for black-purple-curved were subjected to radium radiations for each of the different intervals subsequently noted, and five sister individuals were reserved as controls. A glass tube about 1 by ¼ inches containing the flies was placed in contact with the glass tube containing the radium. The alpha particles and some of the slower beta rays were thus cut off by the glass, but most of the beta rays and all the gamma rays may be supposed to have acted on the flies.[1] Since the flies were constantly moving about in the tube each received an approximately equal radiation. Both the experimentally treated and the control females were back-crossed to black-purple-curved males, and placed in bottles which were changed every four days. Counts of the offspring were made for a period of twelve days only.

[1] *Biol. Bull.*, xxxv, 1. 1918.
[2] *Proc.* Soc. Exp. Biol. and Med., xx, p. 335. 1923.
[3] *Sci.*, lviii, 1494. 1923.
[4] *Jour. Exp. Zool.*, 24, 2. 1917.

The following table gives the percentages of crossing over shown by the different sets of flies for the black-purple region only. This shorter region has been shown to be more "sensitive" to temperature changes than the purple-curved, and the values for the latter region in the radium series parallel the ones given with less significant differences.

SUMMARY OF THE EFFECTS OF RADIUM RADIATION ON CROSSING OVER

Days after mating	Percentages of crossing over for black-purple			
	Control	Radium 20 min.	Radium 20 min. at same time on 2 successive days	Radium 40 min.
1–4	7.60 ± 0.74	5.02 ± 0.95	7.52 ± 0.88	6.86 ± 1.05
5–8	7.58 ± 0.75	4.57 ± 0.62	9.52 ± 2.16	8.49 ± 1.53
9–12	3.60 ± 0.48	5.30 ± 0.96	3.91 ± 0.98	9.50 ± 1.33

In addition to the periods of radiation given a set was exposed to radium for one hour, but this severe exposure had a very marked lethal effect on the eggs laid. The number of flies hatched —less than 100 in 12 days—was too small to give any significant crossing over values. The same effect, to a lesser degree, was noted in the 40 minute experiment. Several tests of flies from these two series showed that they were themselves sterile, as previously reported by Packard.[5]

In general it will be seen that radiation causes an increase in crossing over which is most extreme for the most severe radiation. After a 40 minute exposure this effect appears first between the 5th and 8th day, and shows most plainly in the 9–12 day period. The difference here is 5.9 per cent., while the probable error of the difference is ± 1.41 per cent. The females which received a 20 minute exposure at the same time on two successive days showed an apparent increase in the 5–8 day period, but the probable error is so high as to make this of doubtful significance.

The result of the exposure for 20 minutes is of interest, though the data are too meager to admit of positive conclusions. The values for both the first and the second 4 day periods show a possibly significant *decrease* in the percentages of crossing over. This is especially true of the second value which is 3.01 per cent. less than the control, and the probable error of the difference is .97 per cent. The third value shows a slight but perhaps signifi-

[5] *Jour. Exp. Zool.*, 19, 3. 1915.

cant increase. It would appear that a short exposure to radium radiations produces a decrease in crossing over in those eggs which are closer to the point where the crossing over takes place, but a slight increase in those which are still further back in the early oogonial period.

There is an interesting parallelism between the effects noted above, and the first chromosome data of Mavor following X-ray exposure. I have shown[6] that this chromosome appears to cross over less freely than at least the black-curved section of chromosome II. Mavor and Svenson found that the same dose of X-rays which produced an increase in crossing over in chromosome II caused a decrease in chromosome I. Since the most effective agents in radium radiation are apparently the gamma rays, which are actually hard X-rays, one might venture the suggestion that the same dose of X-rays which produces the marked increase in crossing over in the second chromosome, produces only the initial decrease in chromosome I because it responds less freely. In any case these data make it of interest to know the effect on crossing over of varying amounts of X-ray or radium treatment.

It is obvious that radium exposure corresponds with the X-ray treatment in the fact—pointed out by Mavor and Svenson—that it produces some change in the protoplasm, or the chromatin, of cells which have not reached the oocyte stage. I calculated that approximately 150 eggs were in this stage or later in the ovaries of female flies which had just hatched. The effect of X-rays for 3 minutes or radium for 20 minutes is thus produced on eggs in the oogonial stage, though it does not become apparent until the period of crossing over is reached. It should be noted, however, that temperature also produces an effect on oogonia, for I showed that only eggs which have been exposed to high temperature for at least 24 hours previous to the time of crossing over, show a change in the crossing over percentage.

HAROLD H. PLOUGH

AMHERST COLLEGE

BIRD MIGRATION AND PLUMAGE SUCCESSION CHARTS

AMONG the many complex phenomena of bird life, the distribution and seasonal movements of the various migrational groups within any given area and the annual procession of plumages,

[6] *Jour. Exp. Zool.*, 32, 2. 1921.

with their associated molts, cause, among beginning students of the subject, perhaps the most confusion. The charts accompanying this paper have been used with much success by the writer, with such students.

Within any given area (represented by the stippled portion of Fig. 1) there normally occur six more or less well-defined groups of birds. (1) Permanent residents, or those which apparently do not migrate, at least to any great degree. This group contains such birds as are represented the year around *as to species*, but not necessarily as to individuals, *i.e.*, it is probable that the individuals that one sees in the winter are not the same ones observed during the nesting season, in most cases. Familiar eastern examples of birds of this group are: white-breasted nuthatch (*Sitta carolinensis*), quail (*Colinus virginianus*), downy woodpecker (*Dryobates pubescens medianus*) and hairy woodpecker (*Dryobates villosus*). (2) Spring migrants, or those birds which nest to the north of the area and winter to the south of it, and pass through the area northward in early spring. Examples: white-throated sparrow (*Zonotrichia albicollis*), myrtle warbler (*Dendroica coronata*) and yellow-bellied flycatcher (*Empidonax flaviventris*). (3) Fall migrants, or those birds of the group just mentioned, which are returning to their wintering grounds in the fall. (4) Summer residents, the largest group, consisting of those birds which winter to the south of the area, and return to it each season to nest. This group contains the best known birds of each area, since they pass a longer time within its bounds than do the birds of any other group, beside nesting and rearing their progeny here. Such birds are the familiar robin (*Planesticus migratorius*), bluebird (*Sialia sialis*) and a host of others quite as well known. (5) Winter residents, comprising those birds which nest to the north of the area, and winter within it, such as: Red-breasted nuthatch (*Sitta canadensis*), bluebill, or scaup duck (*Marila affinis*) and herring gull (*Larus argentatus*). (6) Irregular visitants, which are not, properly, members of the avifauna of the area, but accidental visitors, which straggle in and out in an unpredictable fashion. Such an irregular visitant, for our northeastern states, is the evening grosbeak (*Hesperiphona vespertina*). In Figure 1 the spring migration is shown by broken lines, and the fall migration by solid lines. The arrows for the irregular visitant group are intended to indicate that their movements are capricious.

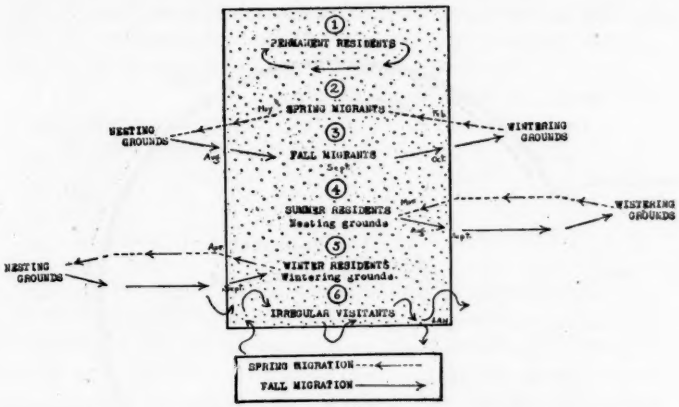


FIG. 1

Chart showing the movements during migration of the six well-defined groups of birds in any given area

For restricted areas, where a careful migration record is being kept, such a chart is very helpful. It can be expanded, and spaces, divided in convenient fashion, left under the name of each group for the listing of the birds of that group, with the dates of their arrivals, departures or nesting.

The plumage and molt chart (Fig. 2) gives the succession of the various plumages and molts for the young and adults of a typical passerine bird. In general these changes among the Passeres occur as follows: When hatched the bird is naked, except for a scanty covering of fine down, the first plumage, termed the natal down. The natal down is quickly molted (first molt, postnatal molt) and the second plumage, juvenile plumage, succeeds. Within about two weeks after acquiring this plumage the bird usually is ready to leave the nest, when there occurs the second molt (postjuvenile molt), which leaves the bird with its third plumage (first winter plumage). This in turn is lost by the third molt (prenuptial molt), which takes place in the early spring. In most cases the bird has now acquired its adult plumage with the first nuptial plumage (the fourth since it left the egg), which in the fall by the fourth molt (postnuptial molt) leaves it with its fifth plumage (winter plumage). Thereafter, year by year, it undergoes but two molts, the prenuptial in the spring, and the postnuptial in the fall; and wears but two changes of plumage, the nuptial in the summer, and the winter

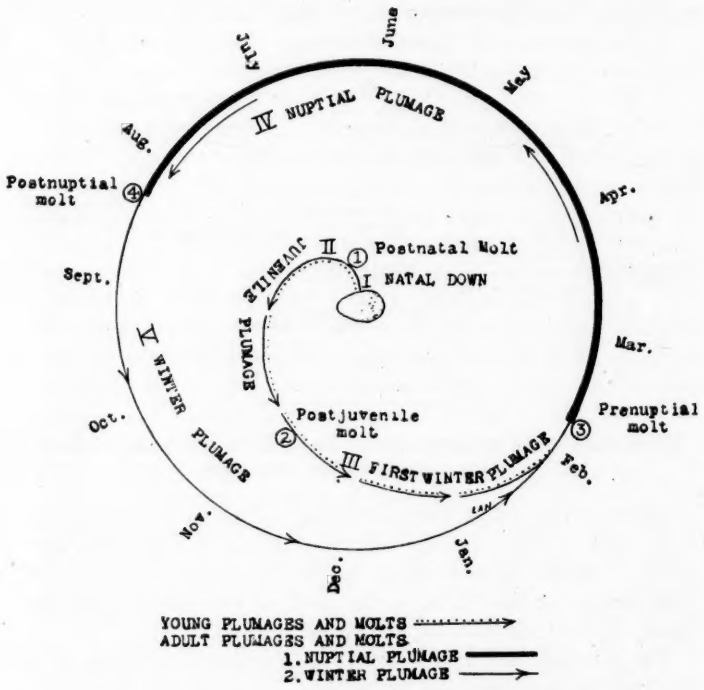


FIG. 2

Chart showing the typical succession of plumages and molts in the young and adults of passerine birds

Plumages in Roman numerals

Molts in Arabic numerals

plumage during the remainder of the year. A multiplicity of specific variations of the above molt and plumage sequence occur among birds, and new plumage colors and patterns are also developed by reason of feather wear, and fading. Such changes are not susceptible of being charted on any general schema, but must be separately recorded for each different species. All birds, however, pass through a complete molt after the breeding season (4, postnuptial molt) after which they acquire the winter plumage. Curiously enough, the males of some birds breed in the immature plumage, as, for example, the orchard oriole (*Icterus spurius*) and the redstart (*Setophaga ruticilla*).

In Fig. 2 the dotted arrows from the egg (center) to the circle

show the succession of immature plumages and molts, and the circle the succession for adults, with the approximate months when the molts occur and the plumages are worn.

LEON AUGUSTUS HAUSMAN

ZOOLOGICAL LABORATORY,
RUTGERS COLLEGE

LUTEAL CELLS AND HEN-FEATHERING

WHEN a hen-feathered male is castrated, as shown by Morgan, cock feathers replace the hen feathers. The change is considered by him to be due to the removal of the secretion produced by certain cells, identical with the luteal cells of the ovary, as one of us (N) has recently shown. While N was making histological studies of the developing gonad, the other (G) had become skeptical of the reputed relation between the luteal cells and hen feathering and as a consequence undertook observations and experiments to determine the relationship. Finally, the following test was made. From a large series of sections prepared in G's laboratory at the Massachusetts Agricultural Experiment Station, twelve were sent to N for examination, he being ignorant of the sort of plumage worn by the birds from which the testes were taken. The testes were from young birds in that stage of development where the kind of plumage could be accurately determined if the birds were to become cock-feathered. Only one testis was removed from each bird, and all were allowed to reach maturity. The result of the test is shown in the accompanying table. Nearly all the birds listed are descended from a hen-feathered hybrid male of Morgan's. G is responsible for the data listed in the first two columns, and N for that in the remaining four. His attempt to determine the nature of the feathering from an examination of the sections was based on the condition of the lymphoid cells, for N already had come to doubt the reputed significance of the luteal cells. It is clearly evident, as an inspection of the table shows, that neither luteal cells nor lymphoid cells can be considered causative agents in the production of hen-feathering.

One consideration remains to be stated. In nearly all adult hen-feathered males, the amount of intertubular material is large, contrasted with that found in all cock-feathered males thus far studied by us, but that this material has any causal re-

TABLE I

Band Number	Actual Feathering	Nonidez's Report			
		Feathering	Luteal Cells	Lymphoid Cells	Condition of the Testes
B8218	cock	.....	none	present	juvenile
B8627	cock	hen	none	present	early spermatogenesis
B8628	hen	.....	none	present	juvenile
B8698	cock	hen	present	present	full spermatogenesis
B8734	hen, later changing to cock	.....	none	present	early spermatogenesis
B8896	hen	hen	none	present	early spermatogenesis
B8908	cock	.....	none	present	full spermatogenesis
B9565	hen	.....	none	few	full spermatogenesis
B9567	cock	cock	present	present	full spermatogenesis
B9705	cock	.....	none	few	full spermatogenesis
C2612	hen	hen	present	present	juvenile
C2615	hen	.....	none	present	juvenile

lation to hen-feathering remains to be determined. Such relation seems improbable, and, like the pigment cells in the sebrights' testis, is very likely a mere varietal difference.

H. D. GOODALE
JOSÉ F. NONIDEZ

INTERTUBULAR TISSUE IN THE TESTES OF CERTAIN BIRDS

THE intimate relationship existing between the gonads and plumage in poultry, coupled with the reference of this relationship to the luteal cells, suggested that a study of the gonads of wild birds should be of value. Plans were made for examining most of our common wild birds but illness restricted the examination to a few specimens, collected and sectioned in 1921.

In three species of warblers, *viz.*, chestnut sided, myrtle and Maryland yellowthroat, very large conspicuous cells, considered by Dr. José F. Nonidez, who examined the slides, to be large lymphocytes, form a tissue in the intertubular spaces. Such cells are absent from the testes of a brown thrasher, several English sparrows and domestic ducks, and are found with difficulty in the testes of a robin and a bluebird. These cells are entirely unlike luteal cells, and much larger than the lymphocytes found in the chicken's testis. Consideration of the plumage of these birds makes it evident that no constant relation exists between the cells described and sexual dimorphism of plumage.

Luteal cells could not be identified satisfactorily in the ovary of an adult robin, nor in the juvenile ovaries of the English sparrow and rosebreasted grosbeak. In domestic ducks they are less conspicuous than in domestic chickens.

H. D. GOODALE

THE ABSORPTION OF THE PUBIC SYMPHYSIS OF THE POCKET GOPHER, GEOMYS BURSARIUS (SHAW)[1]

THE pocket gopher is one of several fossorial animals which has the pelvic girdle greatly reduced, apparently as an adaptation for turning in the narrow confines of its burrow. This reduction has so developed that, in many instances, the puboischiatic-vacuity has become too small for the birth of young and so has necessitated other adaptations. The pubic symphysis of the mole (Talpidae) may be absent or, if present, meets dorsal to the digestive and urogenital tracts (Slonaker, 1920)[2] and the shrews (Soricidae) have no symphysis (Lecke, 1884),[3] while in the Rodentia some of the voles (subfamily Microtinae) also are without a symphysis.

From an examination of 56 female pocket gophers, representing 13 species, Chapman (1919)[4] found that 38 had complete

[1] Contribution No. 68 from the Zoology Department, Agricultural Experiment Station, Kansas State Agricultural College.

[2] Slonaker, J. R., 1920, Some Morphological Changes for Adaptation in the Mole. *Jour. of Morph.*, Vol. 34, pp. 335–365.

[3] Lecke, W., 1884, Mammalia, pelvis. Bronn, Klassen und Ordnung des Tierreichs. Bd. 6, s. 571.

[4] Chapman, R. N., 1919, A Study of the Correlation of the Pelvic Structure and the Habits of Certain Burrowing Mammals. *Amer. Jour. of Anat.*, Vol. 25, pp. 185–208.

pubic symphyses, while in 18 the pubic bones not only did not meet to form a symphysis but were almost absent and spread far apart. He also states that "no intermediate conditions have been found; the bones either meet to form a symphysis or are widely separated. Very young females have been found possessing a symphysis, while old females without a symphysis are not uncommon. It seems, therefore, that the presence or absence of the symphysis can not be a matter of ossification of the bones due to age." In his discussion he further states that "the pelvis is broad and has allowed sufficient room for the passage of the fetuses at the time of birth, and there has, therefore, been no necessity for its loss," and he concludes that "the pocket gophers evidently represent a stage in which the room within the pelvis is greatly restricted and the symphysis is in the process of being lost."

During the last four years the writer has examined over a thousand pocket gophers, *Geomys bursarius* (Shaw), which were collected during all months of the year, and from this material it was found that the development of the pubic bones of both the male and female was identical up to the approach of sexual maturity. The pubic bones are among the last of the pelvis to become ossified and are completely cartilaginous when the animals are two weeks old, while ossification is not complete before they are two thirds grown. During the fall and early winter the ossified symphyses of both full-grown males and females, born during the breeding season of the preceding spring, are complete and similar, but as the next breeding season approaches, when the animals are almost a year old, the pubic bones of the young females begin to be absorbed, first in the symphysis region and then laterally almost to the obturator foreman. All stages in the disappearance of the symphysis have been observed. This absorption is correlated with the general activities of the reproductive system preceding pregnancy and is usually complete before copulation. All of the pregnant females examined had the symphysis completely open, even though the fertilized ova had not implanted, and in no instance has there been any evidence to indicate that the symphysis is restored after parturition. Careful measurements of newly born young show that they could not pass through the pelvic opening if the pubic symphyses were present. These facts seem to indicate that the absorption of the pubic symphysis of the female pocket gopher is an adaptation to compensate for a reduction of

the pelvic cavity which is correlated with the animal's fossorial habits.

Of the 500 adult females examined only three having a complete symphysis were taken during the breeding season, and in these cases one had deformed reproductive organs and those of the other two were in a non-active, juvenile condition. It has also been found that young females having cartilaginous pubes when placed in captivity will develop bony symphyses like those of gophers in the field, but it is difficult to regulate laboratory conditions so that the symphyses will be absorbed. Some pocket gophers have been kept in captivity for over eighteen months without any apparent change in the symphyses. These captive females grow to full size and appear normal in every respect, but an examination has always revealed that in addition to a closed symhyses the reproductive system was in a juvenile and non-active condition. The two horns of the uterus were never found congested and appeared as white bands, while the ovaries were small and smooth, showing no signs of follicular activity.

The pubis of the male pocket gopher is different from that of the female in that it persists throughout life. In an examination of over 500 males one had a small notch separating the two pubic bones. These observations on the males agree with those of Chapman ('19), who states that all of the male specimens he examined had the pubic bones uniting to form a symphysis.

The fact that the female pocket gophers lose their symphyses during the breeding season and that the loss is always associated with active ovaries and uteri seems to indicate that the real causative agent is supplied by some part of the reproductive system. It is also significant to note that this seems to be peculiar to the female reproductive system because, even though the male reproductive organs undergo very marked changes during the breeding period, the pubis is not absorbed. Experiments are in progress to determine the part of the female reproductive system to which the absorption of the symphysis is due, and the data already obtained seem to indicate that the ovaries supply a factor.

It has been found that castrated males lose their symphyses if given intraperitoneal injections of ovarian extracts. Also the time intervening between castration and the administering of the extracts seems to have a direct bearing upon the length of time and number of injections required to cause the male to lose the symphysis (Table I). Males that have been castrated four

TABLE I

SHOWING THE INFLUENCE OF OVARIAN EXTRACT ON THE PUBIC SYMPHYSES OF CASTRATED MALE POCKET GOPHERS

No. of Animal	Date castrated	Date of 1st injection	Roughenings of pubic bones	Notch at symphysis	No. of injections	Time in days from 1st injection
113	6- 5-22	11-17-22	?	1- 1-23	6	45
114	4-19-22	11-17-22	12-17-22	1- 6-23	8	50
144	12- 1-22	12- 7-22	1-15-22	2-19-23	11	74
153	11-29-22	12- 2-22	2- 1-23	2-19-23	11	79
164	11-12-23	11-10-22	3- 5-23	3-19-23	17	129

to seven months (Nos. 113 and 114) before treatment usually lose their symphyses in six to eight weeks when injected intraperitoneally once a week with three cubic centimeters of normal saline solution containing about one grain of desiccated ovarian substance of the sow, while males that are castrated and receive this treatment immediately (Nos. 114, 153 and 164) require from eleven to seventeen weeks to lose the symphysis. It has also been found that adult females having a pubic symphysis lose it if given ovarian extracts, as described for the male, and the time and number of treatments required is about the same as that for males that have been castrated for several months. In both males and females the first indication that the symphysis is being absorbed is a roughening of the pubic bones. This roughening is easily detected by palpation and occurs two to four weeks before a median notch separating the pubic bones is formed at the symphysis.

Work is in progress to determine the effect of ovarian extracts on the smphyses of uncastrated males, both durng the breeding season when the testicles are large and secreting spermatic materials, and out of the breeding season when they are small and do not contain functional spermatozoa. Studies are also being made of the effects of ovarian extracts on the cartilaginous symphyses of young males and females to see if it is possible to cause absorption of the cartilage before it is ossified. The possibility that the ovarian extract has a definite relationship to the calcium balance is also being taken into consideration and a study is being made of the calcium metabolism of both normal and experimental animals.

FREDERICK L. HISAW

KANSAS STATE AGRICULTURAL COLLEGE